ANNUAL REPORT
OF
PROGRAM ACTIVITIES

NATIONAL INSTITUTES OF HEALTH
1968-1969

DIVISION OF BIOLOGICS STANDARDS

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Report of program activities.

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DIVISION OF BIOLOGICS STANDARDS

ANNUAL REPORT

July 1, 1968 through June 30, 1969

TABLE OF CONTENTS

	Serial No.	Page
OFFICE OF THE DIRECTOR		
Introduction (Summary)		i
LABORATORY OF BACTERIAL PRODUCTS (LBP)		
Summary		1
The Composition, Immunology, and Specificity of Tuberculin	100	9
Immunology of the Delayed Contact Sensitivity Induced by Catechols Related to and Contained in Poison Ivy Extracts	102	11
Standardization of Allergenic Extracts	119	13
Standardization of Methods of Skin Testing Humans for Allergy	134	15
Immunology of the Marmoset, Saguinus mystax	159	17
Investigation of Tetanus Toxoids for Prevention of Neonatal Tetanus	12	18
A Study of the Antigenic Substances of Clostridium tetani	13	20
Standardization of Reference Preparations for Tetanus Toxoids	14	22
Investigation of Toxocity of Oil Emulsified Antigens	15	24
Studies on Cholera and Cholera Vaccine	22	26
Studies on Pertussis Vaccine	104	30
Studies on Typhoid Vaccine	116	32
Evaluation of Microbially Controlled Animals in Control Testing and Infection	136	34
Selective Breeding to Establish a "Standard" Mouse	138	36
Studies on Tuberculosis Vaccine	101	38
Hypersensitivity and Specificity of Fungal Diagnostic Substances for Dermal Tests	103	39
Pathogenesis of Lesions Induced by Antigens in Water-in-Oil Emulsions	120	41
Mycobacterial Antigens: Immunological Specificity and Antigenic Analysis	135	42
Delayed Skin Reactivity and Lymphocyte Transformation in Monkeys with Experimental Allergic Encephalitic (EAE)	160	45
Role of Transitional L-Forms of Bacteria in Human Disease	16	46
Mycoplasma in Human Disease and Biologics	17	48
LABORATORY OF BIOPHYSICS AND BIOCHEMISTRY (LBB)		
Detection and Characterization of Viruses	86	52

	Serial No.	Page
LABORATORY OF BLOOD AND BLOOD PRODUCTS (LBBP)		
Summary		61
Preparation, Characterization, Stability and Potency of Blood Products	33	67
The Clotting and Fibrinolytic Systems	81	70
Proteins in Body Fluids	82	74
LABORATORY OF CONTROL ACTIVITIES (LCA)		
Summary		77
Development of Methods for the Chemical Analysis of Biological Products	29	83
Standardization of Coccidioidin	36	85
Bacteriodes vulgatus for Testing Fluid Thioglycollate Medium.	143	86
Clostridium botulinum Toxin and Antitoxin	144	87
Coral Snake Venom and Antivenin	145	89
Standardization of Reference Diphtheria Antitoxin for Flocculation Test	146	91
Development of an Improved Opacity Standard	147	93
Development of Sterility Test Procedures for Testing Dried Smallpox Vaccine for Jet Gun Injection.	148	95
Replacement of the U. S. Standard Tetanus Antitoxin	150	97
Development of a Reference Typhus Vaccine and an Improved Potency Test	153	99
Bactericidal Activity of Wasp Extract	154	101
RIF-free Yellow Fever Virus Seed	155	103
Malayan Pit Viper Venom	168	105
Comparison of Titrations in Monkey Kidney Tissue Cells and Chorioallantoic Membrane of Chick Embryos for the Potency Assay of Smallpox Vaccine	169	107
Standardization of Reference Blood Group Substance A and B.	170	108
LABORATORY OF PATHOLOGY (LP)		
Summary		111
Development Studies of Neurovirulence Testing on Poliovirus Vaccine, Live, Oral, in Monkeys	41	115
The Pathogenesis of Infectious Diseases	44	117
Histologic and Ultrastructural Development of the Central Nervous System and its Relationship to the Immunological Response	45	121
Developmental Studies and Control Testing of Attenuated Live Virus Vaccines	112	123
In vitro Study of Cells from the Central Nervous System	113	126
Studies of Interferon Production.	131	128

	Serial No.	Page
Studies of Marmosets (Saguinus mystax) and their Suitability for Biologics Testing	133	130
Chromosomal Effects of in utero Viral Infections on Sub-Human Primates	161	135
The Effect of Bacterial Viruses on Animal Virus Infectivity and on Animal Cells	162	137

LABORATORY OF VIRAL IMMUNOLOGY (LVI)

Summary		139
Investigations Concerning Rubella Virus and Vaccine	49	146
Studies of Viruses Associated with Infectious Mononucleosis and Human Lymphoma	127	157
Investigations Concerning the Smallpox Group of Viruses and Smallpox Vaccines	47	159
Study and Diagnosis of Heritable Metabolic Disease by Use of Tissue Culture	53	161
Investigations into the Etiologic Agent of Viral Hepatitis	137	165
Investigations Concerning Measles Virus and Vaccine	46	168
Studies on Simiam Virus$_{40}$	52	169
Investigations Concerning Combined, Live Virus Vaccine	126	170
Investigations Concerning Cytomegaloviruses	171	171
Investigations Concerning Respiratory Syncytial Virus	172	173
Investigations Concerning Mumps Virus and Vaccine	48	175

LABORATORY OF VIROLOGY AND RICKETTSIOLOGY (LVR)

Summary		179
Office of the Chief	57	191
Studies on Autoimmune Reactions Related to Viral and Adjuvant Sensitization	139	193
Studies on Agents of Chronic and Degenerative Diseases	140	195
Improved Techniques for Isolation and Study of Viral Agents Affecting the Nervous System	173	197
Studies on Arthropod-borne Viruses (arboviruses)	75	200
Studies on Hemorrhagic Fever Viruses	118	202
Studies on Yellow Fever Vaccine	121	205
Studies on Respiratory Viruses. I. Influenza Virus Vaccines	122	209
Studies on Endogenous Viruses Contaminating Avian Eggs. I. Duck Hepatitis Virus	141	212
Avian Leukosis Viruses in Relation to Human Vaccines	117	214
Egg Yolk Antibody as a Tool to Study Avian Microbial Infections Potentially Contaminating Human Vaccines	125	217
Studies to Elucidate the Cause and/or Etiological Agent Causing Marek's Disease in Chickens	163	219
Studies on Endogeneous Viruses Contaminating Avian Eggs. II. Group Specific Antibody of Avian Leukosis Viruses in Experimentally Infected Pigeons	164	221

	Serial No.	Page
Studies on Smallpox Vaccines and Vaccinia Virus	124	223
Antigenic Studies of Rickettsial Vaccines	59	225
Mechanisms of Intracellular Infection	65	227
Studies of the Nucleic Acids and Genetic Relatedness of Rickettsiae	82	229
Standardization of Techniques Applicable to Large-Scale Production of Cell Cultures	67	231
Development of New Cell Culture Systems as Possible Substrates for Vaccine Production and/or Testing	79	233
Studies on the Biologic of Cultures Cells	83	235
Identification of Specific in vitro cell differential antigens	142	237
Studies on Immunologically Active RNA (Ribonucleic Acid)	165	239
Immune Reactions and Immunochemistry of Tissue Culture and Cellular Antigens	166	241
Immune Responsiveness in Man to Viral Agents	167	243
Studies Concerned with Influenza Viruses	60	245
Studies Concerned with Adenoviruses	98	251

CONTRACT NARRATIVES

Allergenic Extracts	Development of Information on the Activity of Allergenic Extracts (NIH-69-2010)	289
Beta-Propiolactone	Investigation of the Reaction Products of Beta-Propiolactone (PH-43-67-1397)	277
Carcinogenesis	Study of Carcinogenic Potential of 7 Chemicals Used in Biological Preparations (PH-43-67-677) (PH-43-67-676) (PH-43-67-684)	264
Chem. Carcinogenesis	Investigations on the Mechanisms of Chemical Carcinogenesis (PH-43-67-675)	262
Diploid Cell Strains	Development of Sub-Human Primate Diploid Cell Strains and Established Cell Lines (NIH-69-98)	287
Diploid Cell Strains	Development of Sub-Human Primate Diploid Cell Strains (NIH-69-100)	288
Electron Microscope	Electron Microscope Services (PH-43-67-1105)	274
Electron Microscopy	Electron Microscopy Services (NIH-69-2013)	290
Exchange Transfusions	Studies of Patients Receiving Exchange Transfusions in Neonatal Period (PH-43-67-701) (PH-43-67-705)	268
House Dust	Identification, Isolation, and Culture of Mites from House Dust (NIH-69-65)	285
Human Karyotypy	A Statistical Approach to Human Karyotypy (PH-43-67-1463)	278
Inf. Mononucleosis	True Incidence of Infectious Mononucleosis in Science (NIH-69-85)	286
Latent Infection	Studies of Latent Infection and Tissue Culture Phase (PH-43-67-1106)	275

		Serial No.	Page
Latent Infections	Studies of Latent Infections: Tissue Culture and Animal Phases (PH-43-67-1100)		270
Measles Vaccine	Study of Recipients of Attenuated Measles Virus Vaccine Prepared in Canine Kidney Cells (PH-43-68-646)		282
Mycoplasma Antigens	Preparation of Mycoplasma Antigens and Antisera (PH-43-66-1163)		261
Oil Adjuvants	Studies on the Biologic Activity of Oil Adjuvants (PH-43-67-679)		266
Oils & Emulsifiers	Chemical and Physical Analysis of Oils & Emulsifiers (PH-43-67-1101)		272
Oncogenic Activity	Studies of the Oncogenic Activity of Human Biological Products in Syrian Golden Hamsters (Mesocricetus auratus) (PH-43-67-1483)		280
Plastic Containers	Effects of Storage Conditions on Plastic Blood Containers (PH-43-65-1046) (PH-43-68-97)		259
Poison Ivy	Isolation of the Active Principles of Poison Ivy, Oak, Sumac, and other Plants of this Family and the Synthesis of Substances Related to the Active Principles (PH-43-64-76)		256
Poison Ivy	Determination of Potency and Efficacy of Urushiol, the Active Ingredient of Poison Ivy Extract (PH-43-64-885)		258
Poison Oak	Determination of Potency and Efficacy of the Active Ingredient of Poison Oak Extract by Clinical Studies (PH-43-68-632)		281
Pollen Extracts	Studies of the Biochemical Properties of Pollens and Pollen Extracts (PH-43-68-1250)		284
Rhesus Monkeys	Housing and Maintenance of Rhesus Monkeys (PH-43-68-662)		283

ADJUNCTS TO THE OFFICE OF THE DIRECTOR

Experimental Virology	01	292
Biometrics Section		
Summary		295
Development of Statistical Methodology Applicable to Biologics Control	111	301
Investigational New Drugs Office (IND)		303
Contracts Operations Office		
Summary		304
Studies of Interferon Production	131	305
Studies of Virus Cell Interactions	132	307
Investigation of Seven Compounds Commonly Used in Biological Products	174	309
Information Office		311
Scientific Communications Office		313

Page

INDEXES

Contracts 315
Investigators 317
Publications 329

Introduction

SUMMARY OF THE DIRECTOR

INTRODUCTION

The mission of the Division is the exercise of controls on the safety, purity, and potency of biological products through a system of licensing of manufacturing establishments and of the products, as well as the conduct of research related to these objectives in order that decisions made and actions taken can be based on sound information.

Basically, it is the responsibility of the Division to make recommendations for standards for all biological products such as vaccines, serums, human blood and its derivatives, and like products. These include written standards, eventually promulgated as regulations, some of which apply to individual products and some to products in general; and physical standards, i.e., biological preparations whose properties and qualities have been extensively characterized. The latter are issued to manufacturers and other research laboratories concerned with biologics standardization for evaluation with their products, particularly with respect to potency, or strength.

The acquisition of the information needed to develop written standards and the laboratory investigations leading to the development and selection of suitable physical standards are extensive in character and often time-consuming. This is particularly true of standards for the tissue-culture-prepared virus vaccines such as poliovirus, measles, and mumps, which have been developed in recent years.

When a new vaccine against a particular disease appears achievable, it devolves upon the Division to develop standards that will be ready at approximately the same time that the product is brought to the final stages of manufacture. This requires considerable research involving most of the component laboratories of the Division, as well as the testing of samples of both experimental and final products. It is necessary during this period to maintain close contact with the manufacturer and with scientists working in the field so that problems related to standards can be ironed out during the developmental phases. In addition, any clinical investigations conducted by the manufacturers or by investigators studying the products under a variety of conditions come under review of the Division.

When a manufacturer has decided to market a product which he has developed, he submits a license application to the Division together with samples of the product and results of all tests. Division scientists then review the application and examine the product in the laboratory to ascertain the accuracy of the manufacturer's test results. If the tests fulfill the requirements and the application is in order, the product license is recommended for approval.

In the case of a manufacturer already licensed for other biological products, production facilities may be inspected as deemed necessary according to the nature of the pending product. In the case of a new manufacturer, production facilities are inspected and the personnel assessed prior to licensing the manufacturing establishment, as well as the product he proposes to market. The establishment is inspected annually thereafter to verify that proper procedures are being followed in manufacturing and testing. Samples and the results of tests performed on each batch or lot of all biological products proposed for marketing must be submitted to the Division by the manufacturer prior to distribution. The Division checks the test results and runs similar tests to ensure the quality of the products. Only then is a release for marketing issued to the manufacturer.

Once a product has come into use, its safety and potency must be continuously monitored. The knowledge and skill required to do this also provided a sound basis for consideration of problems associated with newer products as they are developed.

Brief reviews of the control activities, and of licensing inspections, investigations, and legal actions are included in this summary. It is noteworthy, however, that except for the staff involved in licensing and investigations, the scientific personnel of the Division's laboratories participate in inspections and control tests as well as in the research which comprises the main substance of this report.

Continued research on both old and new products is necessary not only for obtaining information leading to development of better standards, but also for the development of expertise and scientific competence on the part of the staff. In many respects this is a more compelling inducement towards compliance than mere recourse to regulation-based actions.

Because of the Division's concern with products, as such, and because of the involvement of research staff in the regulatory function, a great deal of attention is directed to "applied" research problems. However, there is probably no organization within the NIH where there are more examples of the failure of the words "applied" and "basic" truly to define research endeavor.

The problems of the Division involve advanced concepts of modern microbiology, molecular biology, protein chemistry, hematology, allergy and immunology. The scientific staff continually studies new methods and new approaches to the problems associated with biological products, because it must remain abreast of current research and be aware of the significance of new knowledge in relation to these products. Of major concern are the viral vaccines, some of which, like vaccinia and rabies, are among the oldest licensed products, while newer ones are continually being developed. The Division must attempt to evaluate the significance of the demonstrated potentiality of some viruses to produce tumors in animals under special circumstances; it must also be concerned with the problems of covert viruses contaminating cell cultures and other systems used for the propagation of viruses. This requires that old products be reexamined in terms of scientific advances from time to time.

Jointly with the National Institute of Allergy and Infectious Diseases the Division arranged for an International Conference on Rubella Immunization at the Clinical Center, National Institutes of Health, in February, 1969. Dr. Saul Krugman of the Department of Pediatrics, New York University School of Medicine, working through a contract between NIAID and the University was the organizer and chairman. The papers presented provided current data on experimental rubella vaccines so that decisions could be made regarding the licensing of rubella vaccines. The proceedings of the conference will be published in the July and August issues of the American Journal of Diseases of Children.

The development of new respiratory viral vaccines, and the continual assessment of older products such as inactivated influenza requires the Division to develop knowledge concerning antibodies involved in serum in comparison with those in respiratory tract secretions, as well as information on the sites of production and on the transport of these substances across cell membranes. Analogous problems exist in regard to the factors possibly related to hypersensitivity which have apparently contributed to severe illnesses resulting from natural infection following artificial immunization with some newly produced experimental inactivated respiratory vaccines, produced from such agents as respiratory virus and Mycoplasma. These are also related to cellular aspects of immunity, hypersensitivity, and allergy, all of which are problems attracting the attention of DBS scientists.

The study of blood proteins also extends beyond their relation to infectious diseases because of the Division's concern with blood and blood products. Work continues on the purification of various plasma components involved in the clotting and fibrinolytic systems.

Bacterial vaccines also continue to be a Division concern. Proponents of pneumococcal "vaccines" are presenting a case that antibiotic therapy cannot work fast enough to save debilitated patients from succumbing to pneumococcal pneumonia. New information concerning the purification of streptococcal M protein point towards the possibility of the development of products for the prevention of rheumatic fever and rheumatic heart disease. Vigorous research on cholera indicates that the likelihood that a toxin, or toxins, produced by the vibrio will be obtained in quantity and serve as the basis for the production of new immunogenic agents. DBS staff members are involved in the cholera investigations and must be prepared for the other developments mentioned above. At the same time, the Division is still confronted with such continuing problems as improvement of pertussis and tuberculosis vaccines.

Another area of current concern and interest to both the Division's and the manufacturers' investigators is that of Mycoplasma. Some of these organisms have been incriminated as causes of disease in man. The research currently being conducted on Mycoplasma is of considerable importance since it has been demonstrated that these organisms grow readily in the cell culture system used for producing many virus vaccines.

Three years ago Division scientists succeeded in attenuating the rubella virus. Using this virus strain they developed an experimental vaccine against rubella or German measles. Infections of this disease during early pregnancy frequently result in spontaneous abortions, still-births or birth of infants with grave defects such as blindness, deafness, heart disease, and brain damage.

Vaccines prepared from an attenuated virus have now been administered to thousands of persons. Over 90 percent developed protective immunity. Continued observation of the first groups of children vaccinated some three years ago indicates that the immunity conferred by the attenuated virus will be of long duration. Thus far, the vaccine appears safe as well as effective.

New techniques of administration of vaccines and other biological products also add to the practical problems of the staff, since these require careful appraisal. In addition, proposals for the use of various oil adjuvants in immunization and in allergy hyposensitization have prompted the Division to mount an extensive series of contractual projects to help resolve the issue of the safety of these materials.

The scientific pursuits of the DBS staff represent responses to several factors. To some extent, the staff must initiate investigations because of proposals for new products being developed by other organizations. To some extent, the control functions lead the staff to recognize gaps in existing knowledge of various biological products and their effects. To a great extent DBS scientists, like their colleagues in other organizations, are motivated by adding to the body of knowledge in their fields.

SCOPE OF CONTROL TESTING PROGRAM

Essentially the control testing program operates as follows: Manufacturers of a licensed product submit to the Division samples from each lot of the product together with protocols containing the results of tests performed on that lot. Some 296 different biological products are currently licensed.

Each protocol is reviewed by the Division to determine if the manufacturer's test results conform to established standards. In addition, some or all of the tests required on a particular product may be performed by the Division as a check on the testing performed by the manufacturer. If the manufacturer's test results and the results of tests performed by the Division are satisfactory, a letter of release for that lot is sent to the manufacturer. Only then may the manufacturer distribute this particular lot for sale.

Two tables are included here to summarize the Division-wide effort. During the 12-month period covered by this report, 4,176 lots of biological products were released, 28 lots were rejected and 44 lots were withdrawn by the manufacturer. Table 1 reflects the number of lots released by categories of products.

A protocol showing the manufacturer's results may consist of considerable information requiring up to 200 pages, for example, on a lot of Poliovirus Vaccine, Live, Oral, or data on only a page or so for a lot of blood grouping serum. All protocols are recorded and reviewed by members of the Protocol Review and Product Release Section. The protocols are then forwarded to other laboratories within the Division for review. The nature of the product determines the Laboratory(s) consulted. A protocol for Measles Virus Vaccine, Live, Attenuated, may be reviewed by personnel in five of the Division's Laboratories.

Some of the Division personnel are involved full-time in the control program, while many others are involved intermittantly as needed according to the field of interest. As the testing of products becomes more specialized, a larger number of the research scientists are becoming involved in control activities.

Table 2 shows the total number of control tests performed by Division personnel last year. This gives no measure of the total effort involved, however, since tests requiring 15 minutes or less are given the same weight as those taking a month or more to complete. Continual attention is given to test development, and progress on these endeavors is to be found in the project reports of the different Laboratories. It is in this area, especially, that staff of the Laboratory of Control Activities work in collaboration with other Division scientists.

The control activities of the Division also requires the maintenance of a large number of different standards and reference materials. These are prepared in the Division or obtained by contract. They are all tested and standardized in the Laboratories of DBS and distributed as needed. During the 12-month period covered by this report 16,072 such preparations were issued.

This illustrates the close relationship which the Division has built up between itself and the pharmaceutical manufacturers in order to maintain the high standards for biological products needed to protect the health of the community. In this respect, the Division again does more than await developments made elsewhere. Industry looks to the Division for the guidelines necessary for improving the quality, and maintaining the safety of biological products.

TABLE 1

Project Group	Lots Released	Lots Rejected	Lots Withdrawn	Totals
Antitoxins	11	0	0	11
Therapeutic Serums	7	0	0	7
Blood and Blood Products	1,454	0	4	1,458
Bacterial Vaccines	165	1	2	168
Toxoids and Toxins	48	0	0	48
Multiple Antigen Preparations	74	3	10	87
Viral and Rickettsial Vaccines	945	6	10	961
Diagnostic Substances for Dermal Tests	135	0	0	135
Diagnostic Substances for Laboratory Tests	1,198	18	18	1,234
Miscellaneous	139	0	0	139
Totals	4,176	28	44	4,248

TABLE 2

TOTAL CONTROL TESTS PERFORMED IN REPORTING PERIOD

Type of Test		Number of Tests
Potency	In Vivo	1,951
	In Vitro	5,004
Safety	General	1,134
	Specific	6,909
Purity		10,415
Sterility		6,278
Total Control Tests		31,691

LICENSING, INSPECTIONS AND INVESTIGATIONS

The Licensing and Investigations Section of the Office of the Director has as its principal responsibility the correlation of licensing and enforcement activities under the Public Health Service Act 42 U.S.C. 262. The following statistics summarize the activities of the units for Licensing, Inspections, and Investigations.

LICENSING

Licensing actions are based on the recommendations of license review committees appointed by the Director from the scientific staff concerned with the particular kind of product or establishment.

As of March 31, 1969, 229 establishment licenses were in effect, together with 126 subsidary establishments and 237 blood bank donor centers. This represents an increase of 9 establishment licenses since April 1, 1968, the date taken for the last report. This difference is due to the issuance of 25 new licenses and the revocation without prejudice of 16 licenses.

During the 12 months covered in this report, 106 product licenses were issued, 77 were revoked without prejudice, and none were revoked for cause. As of April 1, 1969, 1,351 product licenses were in effect for 296 licensed products. Also, during this period, 19 establishment amendments and 74 product amendments were approved.

There were 24 applications and 13 amendments for establishment licenses and 98 applications and 99 amendments for product licenses pending at the time of this report.

Notable among the product licenses issued during the year are two which are new products:

Vaccinia Immune Globulin (Human)
Factor IX Complex (Human)

INSPECTIONS

Forty-two members of the scientific staff, selected by the Laboratory Chiefs and the Office of the Director for their special knowledge, are authorized by the Secretary, Department of Health, Education, and Welfare to serve as inspectors.

These individuals perform inspections as planned and coordinated by the licensing and Investigations Section of the Office of the Director. Seven additional professional employees assist in the inspections.

Between July 1, 1968 and May 1, 1969, 66 of the 78 scheduled inspection trips were completed. During these trips 189 licensed establishments, 91 locations, and 112 donor centers were inspected for a total of 392 inspections. In addition 15 unscheduled trips were taken to inspect 8 establishments for licensing and 13 reinspections.

To accomplish the scheduled inspections, 469 man-days were required and 42 man-days for the unscheduled inspections. The remaining 12 trips will require 87 man-days for 78 inspections.

SUMMARY OF INSPECTIONS FOR FISCAL YEAR 1969

Scheduled Trips Completed	66	Scheduled Trips not Completed	12
Licensed Establishments Inspected	189	Licensed Establishments to be Inspected	38
Locations Inspected	91	Locations to be Inspected	15
Donor Centers Inspected	112	Donor Centers to be Inspected	25
Pre-Licensing Inspections	8		
Man-Days	469	Man-Days Anticipated	87

INVESTIGATIONS

Investigations are conducted by inspectors from the Office of the Director. In contrast to inspections, which call for expenditure of predictable periods of time, investigations must necessarily be carried out for as long and indefinite a period as is required to obtain all the needed information on a reported or discovered possible infraction of the law. Investigations must therefore be carried out by people who have this duty as their main function. Investigators usually require extensive follow-up interviews with many people as the need is indicated in the course of assembling complete and accurate evidence. In some situations, however, it is also necessary to call upon Laboratory resources for assistance, depending either on the product involved or the nature of the alleged violation.

The Investigations Unit's functions frequently overlap those of other Government agencies. For this reason the Unit maintains a continuing liaison with such agencies as the Justice Department, Department of Agrilculture, Customs Bureau, Post Office Department, Federal Trade Commission, and the Food and Drug Administration.

The Investigations Unit is also responsible for acting on reports of hepatitis as they relate to source material used in the manufacture of biological products or to the final product itself. This requires numerous interviews and extensive record reviews to determine the safety of the products involved.

From April 1, 1968 to April 1, 1969 investigations involved a total of 51.5 man-days on travel status. This included visiting both licensed and unlicensed establishments for enforcement purposes and hepatitis follow-ups in addition to interviews and consultations with State officials, distributors of the products involved, employees of investigated establishments, knowledgeable persons in the community and others.

JUDICIAL ACTIONS

The conviction of an officer of a blood bank in Dallas, Texas of having falsely labeled Whole Blood (Human) and Red Blood Cells (Human) was set aside by a federal appellate court in New Orleans, Lousiana on September 6, 1968. The judge based his decision on the determination that these products were not analogous to a therapeutic serum within the meaning of the Public Health Service Act as enacted in 1902. This action has posed a problem to the Division's control over Whole Blood (Human).

The Office of General Counsel has prepared a proposed legislative amendment seeking to revise the description of the categories of products specified in section 351 to state clearly the legislative intent to control under the Public Health Service Act Whole Blood (Human), Red Blood Cells (Human), and Allergenic Products. In addition, to envoke as much authority in this matter, as possible, the Food and Drug Administration has published a notice of rule making proposals to make the Public Health Service regulations part of the Food and Drug Administration good manufacturing requirements to these products in order to insure a legal base for their control by this Division.

VOLUNTARY COMPLIANCE

One voluntary compliance was obtained from an establishment that was shipping partially manufactured blood products to an unlicensed establishment in Europe.

SPECIAL PROJECTS

Blood Donor Study-Atlanta, Georgia.

The analysis of 43,000 microfilmed documents to determine the extent of inaccurate donor identification and too-frequent blood donations is continuing.

OFFICE OF THE DIRECTOR

SUMMARY

Program direction and administration for the Division of Biologics Standards is based in the Office of the Director, with centralization of administrative procedures relating to personnel, supply, and accounting. Major objectives are determined and program responsibilities are assigned to the operating units within the Division. For each major area, staffing patterns are developed and are continually re-evaluated in relation to program changes. The required support is determined for each area in relation to the Division's total budget. A major activity of the Director's office is the maintenance of the proper balance between control activities and research programs.

The Office of the Director includes an Information Office, a Biometrics Section, an Investigational New Drug Office, a Scientific Communications Office, and an Experimental Virology Section.

The Information Office is the means for communicating to the public significant aspects of the Division's research and control activities, responding to requests for information from the general public and for reports to higher echelons within the Public Health Service and the Department, and to Congress.

The Biometrics Section, because of its functions, cuts across the entire Division. The tasks of this section represent the performance of work, using already established statistical techniques, for solving problems related to vaccine-testing originated in various Laboratories. In addition, the Section is involved in studies directed toward perfecting statistical methods used in bioassays and to serving the research needs of the Division. This involves the problems of acceptance sampling and contingency table analysis, relating respectively to the laboratory examination of vaccines for safety and to the evaluation of field trials of candidate vaccines.

The "IND" Office receives and examines "Notices of Claimed Investigational Exemption for a New Drug," which are required by section 130.3 of the Food and Drug Regulations, has continued to function within the Office of the Director. During the period covered by this report, 52 "Notices" were received. This brings to 374 the total number of "Notices" received since the Food and Drug Regulations requiring second submissions became effective. The Office has carried on an extensive correspondence to correct deficiencies in submission and to point out possible hazards. The "Notices" add to the Division's alertness regarding the development of new products. The staff had opportunity to "tool up" in advance for the formulation of regulations and standards for such new products through this mechanism.

Data and information pertaining to licensed biological products and to new products in the offing are of immediate and direct importance to the regulatory and control programs. The Office of Scientific Communications performs bibliographic and reference services for the Division and maintains types of information for members of the staff. It is also working on an effective and more rapid means for processing information. Since the entire field of biomedical research requires new information retrieval techniques, such

developments, for DBS, proceed along with systems that are being worked out for the whole body of knowledge. This requires close contact with information specialists working elsewhere within NIH and in other agencies.

ORGANIZATIONAL CHANGES

Certain organizational changes, directed toward better functioning of the Division, took place during the period covered by this report.
Dr. Roderick Murray, served as Acting Chief of the Laboratory of Virology and Rickettsiology from November 1, 1968 through January 20, 1969 after the departure of Dr. Alexis Shelokov, Chief of LVR on October 31, 1968. On January 21, 1969 Dr. Nicola M. Tauraso was appointed Acting Chief of LVR and this laboratory was then reorganized to reflect the new personnel changes. It is now composed of an Office of the Chief and two sections--Virology and Rickettsiology, and Cell Biology. Dr. Nicola M. Tauraso was designated Chief of the Virology and Rickettsiology Section and Dr. Henry Orr was asked to serve as Acting Chief of the Cell Biology Section. An analytical Chemistry Section was established in the Laboratory of Control Activities.
Dr. Edward B. Seligmann, Jr. is Acting Chief of this Section. The title of the Pyrogens Section in LCA was changed to the Pyrogens Test Section.

PERSONNEL CHANGES

Dr. Alexis Shelokov, Chief of the Laboratory of Virology and Rickettsiology retired October 31, 1968, after 18 years with the Public Health Service, to accept the Chairmanship of the Department of Microbiology, The University of Texas Medical School at San Antonio.

Dr. C. P. Li, Chief of the Section on Virus Biology, LVR, retired October 31, 1968, after some 20 years of Federal service.

Dr. Jose G. Canchola, head of the Respirovirus Unit in LVR, resigned in December, 1968 and Dr. Ruth C. Dunlap, head of the Virus Morphology Unit in the same laboratory, transferred to the Division's Laboratory of Biophysics and Biochemistry in January, 1969.

Dr. Heinz P. Schumacher, a guest worker and an International Post doctoral Research Fellow from Universitats-Kinderklinik in West Germany, is working in LVR's Viral Pathogenesis Unit.

There have been a number of new appointees to the staff. Dr. William J. Davis and Dr. James P. Simsarian, both medical officers, have been assigned to the Laboratory of Pathology. Dr. John M. Nelson, a research biologist, is carrying out research related to the detection of adventitious agents in tissue culture in the Laboratory of Biophysics and Biochemistry.

Dr. Daniel E. Thor, a research investigator, is continuing his studies in the field of immunobiology in the Laboratory of Virology and Rickettsiology. Dr. Thomas C. O'Brien was assigned to the Respirovirus Unit in the same laboratory. Dr. Thomas J. Wera, a clinical associate, was assigned to the Office of Investigational New Drugs.

Dr. Jerome J. Helprin, a biochemist in the Laboratory of Biophysics and Biochemistry, died on June 18, 1968 of a heart attack.

HONORS TO STAFF MEMBERS

Dr. Roderick Murray, director of DBS, was promoted to grade of Assistant Surgeon General on July 19, 1968.

Dr. Harry M. Meyer, Chief of the Laboratory of Viral Immunology, and Dr. Paul Parkman, of the same laboratory received the Department of Health, Education, and Welfare's Distinguished Service Award in recognition of their achievements in the service of the Department; they also received the United Cerebral Palsy's Max Weinstein Award for Medical Research for 1969.

Many other members of the Division have been recognized for their special competences, as indicated by invitations to lecture, to serve as consultants, to serve on committees and study groups, or as leaders of symposia.

CONTRACTS PROGRAM

In 1966, the Division established a contracts program to serve as an extension of, and a supplement to, its research and control activities. Among the principal questions to be investigated was the possible oncogenicity of viral vaccines and the carcinogenic or toxic properties of vaccine components, adjuvants, and other biologics. Since that time, approximately 40 contracts have been initiated to study oncogenicity and other questions pertinent to the Division's role in the development of standard for biological products. Many of these contracts are nearing completion and it is anticipated that significant data should become available during the coming year.

The Division presently supports twenty-four (24) active contracts. It is anticipated that the number of contracts will remain relatively constant as completed projects will be balanced by new project needs.

In those instances where Project Officers are assigned from the individual laboratories to manage the projects, details of each contract which they monitor will be found in the "Contract Narratives." Other projects, initiated and managed by the Contracts Staff of the Office of the Director, are also included in the special Index to Contracts in this report.

Although the contracts staff personnel participate extensively in the supervision of contract operations, providing materials for texts and examining results of day-to-day activities, the scientific staff of the Division also contributes greatly to the development and function of the

contracts program. This contribution has been of particular value in the initiation, supervision and evaluation of the contract efforts. Often this has involved considerable expenditure of time, made at a sacrifice to their personal interests. Members of the contract staff are also engaged in direct research.

RESEARCH ACTIVITIES

The following summary of the program of the laboratories reflects the broad interests of Division scientists. For convenience, the work to be described will be identified only with the laboratory of the principal investigator. There is, however, a considerable amount of inter-laboratory collaboration. (Consult the index to investigators appended to this report.)

SECTION ON EXPERIMENTAL VIROLOGY (OD)

This section devotes its attention to viral carcinogenesis, with particular emphasis on biological factors mediating the extent to which neoplastic changes can be enhanced or suppressed.

The amount of SV_{40} virus injected into newborn hamsters was found to be a determining factor in the number of appearance of tumors. Fewer tumors developed in animals given large inocula than in those which received small doses. These results were obtained when daily inoculations were made over the first 6 to 10 days after birth. They suggest that within this short period of time the larger doses stimulate antibody-production which prevents tumor development.

Apparent suppression of Simiam virus 40 (SV_{40})-induced tumors in newborn hamsters by subsequent repeated injections of one strain of adenovirus 12 is shown. Another adenovirus 12 strain failed to show this suppressive effect under similar conditions. Exploratory experiments of this type have been continued with a number of other adenoviruses. One strain, a type 4, which had never been propagated in monkey kidney cells where it might have been in contact with SV40, appears to have a suppressive effect in the same system. Strains of other types of adenovirus gave equivocal results.

Some evidence was obtained that normal hamsters have a greater resistance to transplanted neoplastic cells than do hamsters already bearing tumors induced by the homologous virus. Transplanted cells induced tumors earlier in the tumored animals than in the normals.

LABORATORY OF BACTERIAL PRODUCTS (LBP)

Tuberculins. The polysaccharide fraction isolated from tuberculin which was as active as the protein fraction PPD in detecting tuberculin sensitivity of guinea pigs was also as effective as PPD in inhibiting migration of cells of sensitive guinea pigs, but not in the induction of blast cell transformation

in sensitized cells and only 20 percent of tuberculosis patients gave positive skin reactions. The patient activity, however, was specific, nontuberculosis patients did not react. Protein fractions remain the diagnostic dermal agent of choice.

Studies directed towards differentiation of tuberculosis from "atypical" mycobacterial sensitivity by means of diagnostic dermal substances have shown (1) a better understanding of the antigenic relationship between the sensitizing species of mycobacteria, (2) a correlation of in vitro lymphocyte transformation with delayed human skin reactivity to test antigens including histoplasmin and (3) a difference in dermal reactivity of PPD and OT licensed products with equal tuberculin unitage in guinea pigs sensitized in several atypical mycobacterial species, respectively. The consensus of investigators, the Tuberculosis Section, NCDC and the Diagnostic Committee, American Thoracic Society, is that the best differential diagnostic dermal test would be the simultaneous use of 5 test units of tuberculin and Battey antigen and that each new batch of tuberculin be characterized for human reactivity in four designated areas of the United States. Implementation will be a long term project. The 1968 Tuberculin Conference, on Evaluation of Procedures for Tuberculin Testing, February 28 & 29, 1968, in Bethesda, provided valuable information and stimulus towards the tuberculin projects.

Tuberculosis Vaccines. A study was initiated to characterize 16 strains of BCG used in different countries for the production of BCG vaccine. The cultures, received through the courtesy of the Trudeau Institute, are showing some marked differences. The study is directed towards determining optimum characteristics of strains for BCG vaccine.

Poison Ivy and Related Extracts. The elucidation of the nature of the chemical structure of the active principle of Rhus plants as delayed contact sensitizers and whether or not desensitization can be effected has been a much more complex problem than anticipated. However, there has been a steady progression of publications of applicable basic information from Contract PH-43-64-76 and LBP investigators. One significant finding this year is that poison oak urushiol has a 17 carbon side-chain and poison ivy urushiol has a 15 carbon side-chain. Currently pure urushiol, supplied by Dr. Dawson, is under clinical investigation to determine if sensitive persons can be desensitized. Preliminary results are encouraging. It is anticipated that this study will establish or refute the claims that poison oak extract is capable of densensitization of man. Rhus plants cause the most frequent and incapacitating contact sensitization illness in the United States. Additional information has been obtained by LBP workers on the dependence of the specificity of the delayed reaction to catechols as well as the immune tolerance to size and shape of the side-chain of the molecule, on the absorption of Pentadecylcatechol and Pentadecylveratrol on protein and their distribution to all internal organs, and on the effect of a series of substituted haloinitrobenzenes on delayed hypersensitization.

Standardization of Allergenic Extracts. Studies have shown that skin reactivity of sensitive persons to short ragweed pollen extracts correlates with the Antigen E content of the extract, determined by the gel-diffusion method, and with the histamine release from the white cells of the patients.

Significant differences were observed between lots of extracts. There was no correlation with protein nitrogen content. Attempts to develop a practical assay for Antigen E in ragweed pollen extracts are in progress. Laboratory and clinical studies are being extended to other pollen extracts. Confirmation of the correlation of laboratory assayed content of pollen extracts with their clinical specificity would provide a breakthrough in the development of specific requirements for the potency of these extracts. Other findings are that storage of short ragweed pollen for 20 years did not markedly alter the quality of the extracts, and that different extracting fluids do not give equivalent extracts. Consistent with the concept that mites in house dust are the cause of allergic activity of the dust, mites have been found in samples of house dust used for preparation of extracts. Mites isolated and cultured will be used to further the study on house dust extracts.

Mycoplasma. Besides contributing a possible harmful element related to vaccines prepared from microorganisms grown in tissue cell culture or chick embryo, mycoplasmata may cause direct effect on the metablism of the tissue cells by depletion of an essential amino acid such as arginine as reported last year. This year in collaboration with LCA it was shown that two species of Mycoplasma promote the yield of a virus by causing a decrease in amount of interferon normally induced by the virus. Factors affecting photo-inactivatio of Mycoplasma have been determined. A new species, M. arginini, was isolated from tissue of several animal species and characterized.

From three of the DBS contracts, new information has been acquired on the mycoplasmal flora of several species of animals used as source of primary cell cultures for propagation of viruses for vaccines as well as on cultural procedures for the isolation and detection of these elusive organisms in those vaccines which are subject to mycoplasma-contamination risk. Knowing what species to search for and how to detect them during the manufacture of a vaccine provides greater assurance of the safety of the product.

Tetanus Studies. The final phase of the collaborative study on the prevention of neonatal tetanus in New Guinea showed that after two primary injections of adsorbed tetanus toxoid a mean protective titer (0.01 μ/ml) was provided for 40 months but not for 54 months. Earlier observations showed that three injections of plain toxoid provided a protective titer for only six months. Antitoxin levels of women injected with one dose of oil emulsified toxoid also declined after 40 months. A higher antitoxin response was seen in women with abscess at site of injection than in those without abscess. Prebooster antitoxin titers determined booster response which was around 10 fold. Again, superiority of adsorbed over plain toxoid for primary immunization is emphasized. Mean antitoxin response to booster adsorbed toxoid was only 1.7 greater than to plain toxoid; no information was obtained on relative duation of response. If confirmed that there is a direct relation between assayed unitage of plain and adsorbed toxoids and the duration of human antitoxin levels, a requirement for quantitative unit assay for tetanus toxoids would be indicated.

The study on the separation and characterization of antigens in filtrates of Clostridium tetani showed the presence of two different esterases, the activities of which were enhanced by human and rabbit gamma globulin and not associated with an immune phenomenon. Antihemolysin was induced in rabbits and monkeys by injection of crude and commercial tetanus toxoids. Activity of certain components of toxin appear to be related to symptoms of tetanus in man other than spastic paralysis. Results may be applicable to standards for Tetanus Toxoid Immune Serum Globulin (Human).

Water-in-oil Adjuvants. In a study to determine factors responsible for sterile abscess formation in recipients of oil emulsified biologics, the use of tissue culture cells continued to show promise. Four of 19 lots of Arlacel A were toxic for cells and three of the four were toxic by the mouse assays; 6 were non toxic by both tests. There was a direct correlation between free fatty acid content and cell toxicity. The cell-culture tests will be used to study toxicity of various diluents and chemically defined components of oil adjuvants that have been separated and studied for activity by other methods.

Typhoid Vaccine. In the study for the development of a potency assay that would reflect the efficacy of various types of typhoid vaccine, focus was placed on the different types of immunological response of three strains of mice to Vi antigen as a component of whole cell vaccine, and Vi fraction in solution or absorbed to erythrocytes. Vi hemagglutinin, bacterial agglutinin and active cutaneous anaphylaxis responses differed with reference to the physical state of the Vi antigen and between these immunological responses and the protective response of mice. Although the protective response (ED_{50}) differed between mouse strains, two gave the same relative potencies between antigens. Another strain gave a significantly larger difference. This could explain differencies in relative potencies of vaccines obtained between laboratories. Vi antigen isolated by a new procedure was significantly more protective for mice than Vi antigen prepared by a published method.

Pertussis Vaccine. The research program on pertussis vaccine was continued towards elucidation of the reactive factors of Bordetella pertussis, and means for laboratory detection and assay of these factors in vaccines, and the relation of the factors to untoward reactions by vaccinated children. Special emphasis was placed on the newly described lymphocyte-promoting factor. The laboratory testing phase of the DBS-Pharmaceutical Manufacturers Association cooperative study has been completed and results are ready for statistical analysis. The clinical phase is yet to be initiated. It has been shown with use of the Rhesus monkey, as others have shown with use of other animal species, that pertussis vaccine accelerates the onset of experimental allergic encephalomyelitis. No relation of this reaction to the so-called pertussis vaccine encephalopathy has been established.

Cholera Vaccine and Toxins. This study is closely tied in with the vaccine field trails and bacteriological studies of the Pakistan-SEATO Cholera Research Laboratory, Dacca, East Pakistan and with cholera investigations of other workers in order to obtain basic information applicable to composition and control testing of protective cholera antigens. The FY 69 field trials showed that monovalent Ogawa vaccine did not protect against Inaba infection whereas the Inaba vaccine afforded significant protection. The results confirm this Laboratory's potency data of the vaccines.

One of the several investigators on the separation of the cholera toxin from somatic antigens has developed what appears to be a breakthrough method that will permit large volume production of toxin for preparation of a toxoid for field study not later than 1971. This Laboratory is currently investigating the separated toxin for the presdnce of somatic antigens, methods of detoxification, immunological response of animals to toxin and toxoid and an in vitro method for titration of antitoxin response that would be practical for the field study.

A correlation between vibricocidal titers and resistance of man to cholera has been established by PSCRL. However, vibriocidal antibodies are found in persons outside an endemic cholera area. A cross antigenic relation observed by Gallut between somatic antigens of Brucella and V. cholerae has been confirmed. In addition, it was shown that sera of convalescent brucellosis patients have not only high and long-lasting vibriocidal titers against V. cholerae, particularly the Inaba type, but pronounced passive protective activity against mice challenged with V. cholerae (with Dr. Gangarosa).

Vibrio cholerae gastrointestical infection of mice. With the view that gastrointestinal flora may be responsible for variable natural resistance of man to V. cholerae, the role of indigenous microflora on resistance of mice to this infection is being studied. V. cholerae was established (without cholera symptoms) in the GI tract of germ-free and gnotobiotic mice infected with either of two lactobacilli, one enterococcus and one bacteriodes, singly or in combination, and housed in an isolator but not when housed in a barrier room environment. Attempts are being made to find the presence of other bacteria such as the fusiformers as the cause of resistance of the latter mice.

Laboratory test animals. The influence of the animal species, the difference between strains of a species and the intestinal microbial flora of animals on the nature of the immunological and toxic response in the evaluation of biological products does not have widespread recognition. Growing out of the significant differences in the response of mouse strains to pertussis vaccine, a cooperative project with DRS of selectively breeding out of the NIH strain a strain with uniform desirable response to pertussis vaccine was initiated and is progressing satisfactorily. Another project emphasized the importance of the mouse strain in determination of the potency of typhoid vaccine in relation to human efficacy.

With the increasing production of microbially controlled mice, it is important to determine the effect of the intestinal flora in control testing. Previous reports showed that germ-free and specific pathogen free mice were so much more resistant to pertussis vaccine than conventional mice that they could not be used for toxicity testing. The effect of microbial control mice on potency assay of vaccines is under investigation.

Basic information about the immunological response of the marmoset was obtained in collaboration with the Laboratory of Pathology. The small size of this primate makes it attractive as a test animal for biologics but there is little information about its immunological response as compared with the monkey species commonly used.

LABORATORY OF BIOPHYSICS AND BIOCHEMISTRY (LBB)

Studies this year were designed to correlate the physical and biological properties of viruses, to develop more specific and sensitive methods for detecting, identifying and classifying viruses, and to study the *in vitro* interaction of recently isolated canine and simian cell adventitious agents with human cells. These studies involve, primarily abortive virus-cell interactions, such as infectious canine hepatitis virus and simian cytomegaloviruses in human cells. The incidence, identity and characteristics of adventitious agents occurring in primary cell types used in live vaccine production were the main targets of study.

As an aid in recognizing the presence of small quantities of viruses and identifying them by electron microscopy, a simple method for pelleting virus-infected cells or cell-free virus in the tips of small, pointed, commercially available plastic capsules has been developed. Virus material which is deposited at the tip of the capsule, remains in a fixed position throughout fixation, dehydration, plastic embedding and sectioning. This procedure eliminates many of the mechanical problems previously associated with pelleting and plastic embedding, and allows excellent results when quantities of viral material are small and when virus concentrations are low. The procedure was successfully used as an aid in identifying viruses occurring naturally in fetal calf serum, duck cell cultures and in primary African green monkey kidney cell cultures.

Twenty-seven of fifty-two tissue culture lots of primary African green monkey (AGM) kidney cell cultures were found to contain monkey cytomegalovirus (CMV). The cell cultures required long incubation periods before CMV could be detected. Less than half the contaminated cultures showed cytopathic effects before four weeks. About one-fifth of the contaminated cultures failed to show CPE during a 40-day observation period; CMV was initially recognized in these cultures by fluorescence microscopy.

Host spectrum experiments carried out on African green monkey cytomegalovirus included the following cells: human, AGM, and rhesus fibroblasts; primary human embryonic kidney (HEK), AGM kidney, and rabbit kidney (RK); human amnion (HA); duck embryo (DE); and BHK-21. After several days incubation, infectious virus was detected in the human, AGM, and rhesus fibroblast and the primary HEK, AGM, and RK. Best virus growth occurred in human lung fibroblasts. Human amnion, DE, and BHK-21 appeared to be "dead-end" hosts.

Thirty dogs were obtained from the NIH Animal Farm. These dogs were selected from a group of several hundred animals on the basis of their general poor health (many were acutely ill). Selection of diseased animals was made in order to increase the likelihood of isolating viruses from their organs. Primary kidney cell cultures were obtained from all thirty dogs, liver cultures were obtained from twenty-nine and pancreas cultures were obtained from two animals. Cytopathic

effects (CPE) developed in many cultures; the remainder were held for a minimum of thirty-five days before examination by fluorescence microscopy and electronmicroscopy. The study is incomplete, but fluorescence microscopy gave positive results in about four-fifths of the cultures; electron microscopy revealed viruses in about two-thirds of the cultures examined. Preliminary results indicate the presence of adenoviruses, herpesviruses, myxoviruses, and two unclassified agents.

Similar studies are being conducted with tissue and eggs from a variety of ducks.

Together with Dr. Lewellys F. Barker studies of the "Australia antigen" have been initiated. Recent reports on the relationship between Australia antigen and human hepatitis led to the study of Australia antigen as a possible etiologic agent of hepatitis and possible indicator of infectious material.

The "Australia" antigen appears to be a virus-like particle on examination by electron microscopy and the antigens found in serum hepatitis, infectious hepatitis and in nonhuman primate blood were indistinguishable by both morphologic and immunologic criteria.

Antibody to "Australia" antigen was found in 8 of 29 hemophiliacs who had been treated in a routine manner with multiple transfusions, and in 2 chimpanzees which had been transfused repeatedly with chimpanzee blood

The 20 mμ particles associated with Australia antigen in the plasma of hepatitis patients have a density in CsCl of 1.27 to 1.28 after lipid extraction. Plasma which had been stored for 17 years at -20°C. contained particles with the same density after lipid extraction. EM examination revealed spherical virus-like particles with a 2 mμ outer coat which is removed by ether treatment.

LABORATORY OF BLOOD AND BLOOD PRODUCTS (LBBP)

The research program of the Laboratory embraced several major areas including the preparation and characterization of blood products, such as those of the coagulation and vibrinolytic systems, investigations of their stability and potency, and standardization and improvement of assay techniques. Products to which attention was directed included immunoglobulins and related preparations, grouping and typing sera, albumin, plasminogen, and Factors I, II, VIII, IX, X, and XIII of the coagulation system.

Ultracentrifugal studies carried out after storage for up to 2 years confirmed preliminary findings that proteolytic degradation of Immune Serum Globulin (human) is greatly reduced, but not eliminated, by heating at 45°C or 50°C prior to storage. Short term investigations revealed that whereas heating at 50°C for 72 hours produced changes readily detectable by electrophoresis or complement fixation, changes produced by heating at 45°C were minimal. Heating at either temperature resulted in a marked decrease in the amount of immunoglobulin dimer present.

Blood Grouping Sera stored for 4.5 years at 5°C exhibited no change in titer. This phase of the study was therefore terminated since 4.5 years exceeds the longest possible dating period. However, since evidence concerning the stability of such sera in the frozen state is conflicting, the portion of the study dealing with frozen sera will be continued.

Tests carried out on NIH reference antisera and commercially prepared grouping and typing sera showed that reliable test results, comparable with those reported by manufactures, could be obtained with Auto-Analyzer(R). Optimal levels of bovine albumin and polyvinlypyrrolidone were determined for the test systems studied; the level of bromelin could be varied over a wide range without affecting the results. These findings demonstrated the capability of the Laboratory to evaluate the potency of such sera, but indicated the need for multichannel analysis if routine testing is to be technically feasible.

Certain of the factors affecting the radioimmunoassay for the potency of $RH_o(D)$ Immune Globulin (Human) were investigated. Information resulting from these investigations was shared with manufacturers of this product in a series of demonstration sessions. A simplified single-dilution potency test was developed. It had the following advantages: 1) only 1 ml of test material was needed; 2) simple hand calculations replaced the computerized data processing; and 3) four to six lots of $RH_o(D)$ Immune Globulin could be analyzed simultaneously for antibody concentration.

Immune Serum Globulin (Human) of placental origin was screed for contamination with blood group specific substances A and B. The degree of contamination was measured serologically by hemagglutination inhibition. Lots from various manufacturers contained from 10 to 200 µg/ml of B substances and from 1 to 20 µg/ml of A substance. Lots of mixed (venous plus placental) origin contained B substance at levels ranging from 1 to 50 µg/ml, whereas lots of venous origin were free of blood group substances.

In studies of the coagulation system, human Factor X was found to have a sedimentation coefficient of 3.7 S and a diffusion coefficient of 5.5×10^{-7} cm^2/sec. Activation of human Factor X in the presence of human tissue was found to yield two active fragments and three or four inactive fragments. A similar pathway appeared to be followed when the activation was carried out in the presence of citrate. Gel filtration on Sephadex(R) G-200 was capable of separating human Factors II and X, Factor X being eluted somewhat earlier. This result was obtained with purified preparations as well as with whole plasma, thus indicating a difference in molecular volume between these two proteins in addition to those differences demonstrating by other methods.

The potential Factor IX reference preparation has been distributed in cooperation with the International Committee on Haemostasis and Thrombosis, to a number of laboratories. Some of these have already reported the Factor IX level of this preparation in terms of the level in a population of normal donors. A tentative assay for Factor IX has been achieved. It was found to yield results which were internally consistent but not necessarily in agreement with those obtained by other laboratories employing different methods. Chromatography on hydroxyapatite, which was proven successful in separating human Factors X and II, resulted in no separation of Human Factors X and IX.

A collaborative study, in which the World Health Organization participated, was carried out to compare the values obtained by tests for Factor VIII as performed in various laboratories. Samples of the new reference preparation were distributed and the reported results were compiled and compared with those obtained in this Laboratory. Factor VIII levels reported by most groups which tested the new reference preparation agreed with those determined in this Laboratory. The preparation therefore may be considered to be satisfactory for use in inter-laboratory standardizations.

In order that the purity of plasminogen purified in this laboratory could be compared with that isolated elsewhere, a detailed examination was made of certain aspects of the casein assay, including the substrate and the units employed. The procedure which was developed in this Laboratory (Annual Report 1964 to 1965) for removal of β-casein by precipitation in the presence of urea was found to vary in effectiveness with the commercial casein used as starting material. The treatment generally increased the sensitivity of the casein as a plasmin substrate, but often failed to eliminate the lag period. Nevertheless, by suitable modification of the assay, a constant ratio was obtained between the activity of streptokinase-activated plasminogen (prepared in this Laboratory) and that of a reference preparation of plasmin, regardless of the casein used as substrate. The modified assay thus appears reliable, is not dependent on a single source of substrate, and yields values which can be compared directly with those obtained in other laboratories.

In conjunction with the National Heart Institute, a chemical test was developed for detection and quantitation of ε-lysyl cross-links in proteins. It consisted of cyanoethylation followed by acid hydrolsis and analysis for lysine. Under these conditions ε-amino-crosslinked lysine yields free lysine whereas lysine elsewhere in the protein molecule yields an N-carboxyethyl derivative. This test and a previously developed enzymic test for ε-(γ-glutamyl)lysine crosslinks were carried out in parallel on a series of samples including: fibrin

contaminated with Factor XIII, fibrin free of Factor XIII, fibrin free lysine measured by the chemical test agreed with those of ε-(γ-glutamyl)-lysine isolated after enzymic hydrolsis; they were greatly depressed in fibrin free of Factor XIII and were restored when Factor XIII was added. This agreement indicated 1) that lysine is not crosslinked to any acceptor other than glutamate and 2) that the cyanoethylation technique is a valid procedure for detecting ε-lysyl crosslinks in proteins.

LABORATORY OF CONTROL ACTIVITIES (LCA)

The Laboratory of Control Activities has prime responsibility within the Division for performing tests on biological products to determine their conformance to standards of safety, purity, and potency. The Laboratory is composed of four sections: Analytical Chemistry, Control Test, Pyrogen Test and Reference Standards.

The Laboratory has the following functions:

(a) To conduct chemical, sterility, general safety, pyrogen, residual moisture and potency tests on biological products submitted for release or in support of license applications. Of all the various licensed products, the number tested by the Laboratory is 270 for sterility, 180 for safety, 90 for potency, 80 for chemical composition, 40 for pyrogens, and 20 for residual moisture. Testing is accompanied by review of the manufacturers' protocols with respect to the tests performed by the Laboratory. Research is conducted on the development and modification of control tests on biological products.

(b) To develop, maintain and distribute material biological standards, references, control materials and reagents necessary for the testing of products to determine compliance with standards. A culture collection is also maintained to support the technical effort of the Division as well as to support control activities as they affect the licensed manufacturers.

(c) To maintain under review published standards, minimum requirements, technical memoranda and other guidance issued by the Division to manufacturers concerning licensable biological products, to develop or propose revisions in regulations when indicated, and to prepare technical specifications and other regulatory procedures for new products as they are developed.

(d) To maintain a close liaison and working relationship with other laboratories of the Division and where indicated with the other Institutes of NIH as well as other outside agencies to insure a continuous flow of knowledge and technical information deemed vital for the proper exercise of control measures of both those products already licensed and new products being developed for license.

(e) To participate in and perform annual inspections of designated license establishments in accordance with specified procedures and specific objectives as prescribed by law.

(f) To participate in and give technical support to investigations performed by the Division in connection with Public Health Service Act.

(g) To participate in the review of applications and reports of manufacturers and their products to determine eligibility for license.

(h) To participate in and give technical support to personnel training programs organized within the Division of Biologics Standards.

Individual lots of licensed products are controlled by the Division through a system of evaluation and release utilizing data submitted by the manufacturer and those developed by the testing program of Laboratory of Control Activities and other testing areas of the Division. All testing is directed toward the objective of assuring that products meet prescribed standards of safety, purity, and potency.

Included in the purity testing of biological products are tests for pyrogenicity, residual moisture and specific chemicals, which also serve as means for evaluating and controlling the safety and stability of such products.

The types of products tested by the Laboratory include antitoxins and therapeutic immune serums, blood derivatives, bacterial vaccines, toxins and toxoids, multiple antigens, viral and rickettsial vaccines, diagnostic substances for dermal tests, antivenins, venoms, bacterial enzymes and allergenic extracts.

In addition to the control testing of biological products within the Division, the Pyrogen Test Section of the Laboratory of Control Activities provides regular cooperative testing services to other NIH facilities such as the Clinical Center Pharmacy, Clinical Center Blood Bank, Media Department, National Institute of Allergy and Infectious Diseases, and the National Cancer Institute; and the Control Test Section performs sterility tests for the Clinical Center Pharmacy and Blood Bank.

Over 10,612 tests were performed by the laboratory during this 12 month period (April 1, 1968 through March 31, 1969) in making the determination that products meet prescribed standards.

An integral part of the system of manufacture and control of biological products is the application and use of material standards. By the use of such standards the potency of a product can be determined and standardized so as to give increased assurance of consistency from lot to lot. It is the function of the standards program of the laboratory to provide these standards and reference preparations so that the control program of the Division as well as manufacturers and others engaged in testing may have available the tools to calibrate and evaluate products. It is largely through use of these preparations that those portions of the biologics control regulations relating to potency can be applied.

In order to maintain an adequate supply of material references and standards it was necessary to prepare and calibrate during the past year the following lots of materials: 13 antitoxins, 1 serum, 2 vaccines, 2 viruses, and 2 skin test antigens. In addition to these materials prepared by the laboratory

the following lots of materials were purchased from commercial sources and finished by the laboratory: 2 antitoxins, 1 serum, and 6 vaccines. A total of 1,058 tests was required to complete a satisfactory standardization of these materials and maintain surveillance of the stability of all preparations. These tests included such procedures as flocculation reactions, animal protection tests, animal titrations, neutralizations and assorted *in vivo* and *in vitro* tests on specific specialized materials.

The technique for freeze-drying is a key procedure applied in the preparation of standards and reference materials whenever possible in order to increase assurance of stability over long periods of storage. Over 7,000 ampules of various preparations were dried during the year.

A total of 988 separate shipments of standard and reference preparations were distributed to manufacturers, PHS and state health laboratories, universities, foreign manufacturers holding U. S. licenses and qualified foreign laboratories (primarily through the World Health Organization).

A culture collection of etiological agents, i.e. viral and bacterial organisms, used in the manufacturing and testing of biological products, is maintained. Approximately 100 different standards, references, and related control preparations are maintained for distribution.

In addition to the effort outlined above, the laboratory staff also participated during the year in a number of ancillary activities directly related to control which accounted for a substantial segment of its working time.

(a) Members of the professional staff of the laboratory participate as members of DBS committees appointed to review, evaluate and recommend appropriate action with respect to new or revised products and establishment license applications submitted to the Division. Such review encompasses detailed scrutiny of manufacturer's production protocols for scientific accuracy and conformance to prescribed standards, testing of the products, inspection of manufacturing facilities, preparation of reports, and final recommendations. Laboratory personnel were members of 15 such committees during the year.

(b) The laboratory staff participated in 25 of the on-site inspections required to be performed annually on all licensed establishments. Approximately 28 man days of work were consumed during the year in this effort.

(c) Members of the staff participated in drafting and revising regulations and additional standards. Redrafts of technical recommendations for the manufacture of Smallpox Vaccine, Measles-Smallpox Vaccine, Typhoid Vaccine, and Tuberculin were prepared. Drafts of recommendations for manufacture of Rubella Vaccine were prepared.

The Laboratory of Control Activities works closely with the biological standardization program of the World Health Organization. During the year it has participated in collaborative studies on the Proposed Second International Standard for Tetanus Antitoxin, the Proposed International Reference Preparation for Cholera Vaccine and the Proposed International Plain and Adsorbed Tetanus and Diphtheria Toxoid Preparations.

Periodically the International Reference Preparation for Rabies Vaccine (prepared by the Laboratory of Control Activities in 1963) is tested for potency by the laboratory in order to evaluate its stability. The Chief of the Laboratory served as a consultant to the World Health Organization in Geneva, Switzerland, and participated in the preparation of a draft of the International Minimum Requirements for Snake Antivenins.

While the primary function of the Laboratory of Control Activities is concerned with various aspects of controlling the safety, purity and potency of biological products, a substantial research program is conducted in order to provide a sound basis for the testing program and selection of preparations for use as official standards and references. The following are summaries of some of the research conducted by laboratory personnel during this fiscal year.

(a) Standardization of Coccidioidin: Studies have been initiated to develop a satisfactory laboratory assay for coccidioidin and to develop an improved reference preparation. Preliminary data indicate the feasibility of quantitative measurements of skin reactivity to antigen in sensitized guinea pigs.

(b) Clostridium botulinum Toxin and Antitoxin: In the study of the relationship of nutrition to biochemical structure of toxins from various types, several toxin preparations have been produced and a number of laboratory techniques modified to be more directly applicable to the objectives of this study. Work is progressing at a constantly excellerating rate.

(c) Coral Snake Venom and Antivenin: Work has continued on the comparison of electrophoretic patterns of venom from several species and subspecies of coral snakes. A manuscript has been prepared for publication. Comparisons of homologous and heterologous venom neutralizing activity of several hyperimmune sera and antivenins are in progress.

(d) Opacity Standard: The U. S. Opacity Standard used in the standardization of cell concentrations of bacterial vaccines and of bacterial challenge suspensions used in potency tests is composed of finely divided glass particles suspended in water. During use and storage the opacity of the standard gradually lessens due to changes in particle size and adherence of particles to the walls of the container. Another undesirable feature is the difficulty of reproducing exactly the properties from lot to lot. For example the present international preparation differs from all previous international preparations as well as from the U. S. Standard. This is causing serious problems in laboratories throughout the world. It has been reported that a stable opacity preparation can be prepared by suspending finely divided titanium dioxide in a transparent solid resin. We have initiated this project to determine if such a preparation is feasible for use as the U. S. Opacity Standard. In addition the use of finely divided glass in resin is being studied.

(e) Smallpox Vaccine: Studies associated with problems in the control of smallpox vaccine have been directed toward dried smallpox vaccine intended for administration by the jet gun. Because a portion of the vaccine is injected below the skin by the jet gun, it has been considered advisable to require that smallpox vaccine intended for jet injection be free of viable bacteria.

When present in relatively small numbers in smallpox vaccine, bacteria are difficult to detect. Therefore, studies have been initiated to devise sensitive techniques for determining the level of both viable aerobic and anaerobic bacteria in smallpox vaccine. These tests will then be applied to the control testing of vaccine.

Data are being collected using the Reference Smallpox Vaccine to estimate the variability of titrations on the chorioallantoic membrane versis in monkey kidney tissue cell culture. The correlation of these assays with skin reactivity is under investigation.

(f) U. S. Standard Tetanus Antitoxin: The supply of the dried master used in the preparation of the U. S. Standard Antitoxin is nearly depleted. This dried preparation was adopted in 1907 and has been in use since that data. Five lots of equine tetanus antitoxin and one lot of tetanus immune globulin (human) are being studied in order to select one having suitable properties for use as a replacement for the master preparation. Work to date indicates that avidity of these preparations can be changed depending upon the type of toxin used.

All antitoxins tested continued to neutralize more toxin up to 48 hours after preparation of the toxin-antitoxin mixtures. Toxin solutions used in these studies did not begin to loose to toxicity until after 48 hours. A glycerinated solution at 5 units-ml (the strength at which the Standard is distributed) of one of the candidate lots was considerably less stable than solutions prepared from the other lots. This lot lost 20% in four months and 35% in six months of storage at 37°C. By 20 months this lot has lost 50% of its activity. Solutions of all lots were stable at 4°C for at least 31 months. In addition this study and testing performed in the laboratory on lots submitted for release indicates that tetanus immune globulin (human) differs from tetanus antitoxin (equine) in the nature of the toxin-antitoxin neutralization reaction which occurs. Studies are in progress to investigate these differences. One obvious difference is in the type of antigen used for immunization of the animals. In the manufacture of tetanus antitoxin horses are hyperimmuned with relatively crude toxin following preliminary doses of toxoid. For tetanus immune globulin human subjects are hyperimmunized with toxoid of relatively high purity.

(g) Malayan Pit Viper Venom: It has been reported that a fraction of the venom from Malayan pit viper (Agkistrodom rhodostroma) possesses anticoagulant and thrombolytic activity. A few clinical studies have been reported as being encouraging. On the basis of these reports a study of the venom has been undertaken in order to provide basic information for eventual evaluation of license applications and control of the product. Three fractions have been prepared and are being studied.

LABORATORY OF PATHOLOGY (LP)

The Laboratory of Pathology has a continuing program to study the pathogenesis of infectious diseases using animal models. This program is designed to meet the overall needs of the Division in regard to the safety

and efficacy of new biological products and also those which have been licensed for many years.

The program of testing the neurovirulence properties of Poliovirus Vaccine, Live, Oral, has centered on the completion of a collaborative study involving the control authorities in England and West Germany as well as the Laboratory of Pathology, DBS. The results indicate that all three laboratories can identify lots of vaccine of clear-cut increased neurovirulence but that technique of inoculation is most-important in the study of vaccines of neurovirulence which is minimally increased.

This year, as last, an extremely low level of poliomyelitis was reported in the United States and in the world. The high level of immunization has propably been responsible for the fact that the number of lots of vaccine submitted for testing was very small. However, the laboratory does perform neurovirulence tests on all of these lots at the present time. The importance of such continued testing was emphasized at a recent World Health Organization conference on Poliovirus Vaccine to which the Chief, Laboratory of Pathology, was a consultant. At this meeting it was stressed that extreme care must be exercised continuously in order to maintain the safety of the vaccine.

Much of the effort of the laboratory is now being directed toward research in, and the development of, significant test systems for the newer attenuated viral vaccines and improvement of test systems for the older viral vaccines.

Yellow Fever Vaccine (17D strain) has been under study in collaboration with the Laboratory of Virology and Rickettsiology and the Laboratory of Control Activities. The neurovirulence properties of this virus have been found to be stable even when a virus of the avian leucosis complex has been removed from the seed virus pool. Since Yellow Fever is a disease that affects the central nervous system as well as the viscera, and since the vaccine has neurotropic properties for rhesus monkeys, control of the stability of neurotropism at a low level is essential for use in this new and more purified vaccine in man. The immunogenicity of this derivative of the primary seed strain and its stability are being investigated.

In collaboration with the Laboratory of Viral Immunology, studies of wild and attenuated rubella virus both in pregnant and nonpregnant rhesus monkeys have been completed. The virus can infect these animals and multiply, although no clinical syndrome developed in either the adult or the fetus. The safety tests for Rubella Virus Vaccine, Live, have been based on these studies and the Additional Standards promulgated.

In collaboration with the Laboratory of Virology and Rickettsiology and Dr. Kendall Smith, studies of wild and "vaccine" strains of human adenovirus 4 are complete. Attempts to infect monkeys appear to be unsuccessful but the virus apparently does cause an inflammatory reaction when placed directly within the central nervous system. This reaction can be found whether crude or purified virus is used.

The Section of Histopathology has greatly expanded its activities in the field of electron microscopy and has developed excellent techniques. Studies of the ultrastructure of the central nervous system are designed to complement similar studies using light microscopy. As the Section has perfected its techniques for ultrastructural studies, it has developed many collaborative programs. The Section of Histopathology has continued to perform clinical laboratory techniques and operates an automatic counter for assaying red and white blood cells. This has enabled the technical staff to perform such procedures with great efficiency.

Dr. Clark has expanded his studies of myelinization of the central nervous system of animals to include studies of demyelinating diseases, such as experimental allergic encephalomyelitis, and has initiated a study of congenital deformities of the central nervous system of animals. Such a baseline study is essential for subsequent evaluation of teratogenesis in relationship to the use of biological products.

Studies by Dr. Clark, in collaboration with the Laboratory of Bacterial Products, have shown that pertussis vaccine has a specific adjuvant effect upon the induction of experimental allergic encephalomyelitis in a number of inbred strains of mice have been unsuccessful.

Further, Dr. Clark, in collaboration with the Surgical Neurology Branch, NINDS, has developed an experimental model for a debilitating abnormality of the central nervous system, namely, hydrocephalus. These studies will be expanded in order to evaluate the possible role of viral infections in the development of such an abnormality.

The Section of Animal Testing, in addition to performing neurovirulence tests on all lots of Poliovirus Vaccine, Live, Oral, and selected lots of Poliovirus Vaccine, live, Oral, and Selected lots of Poliomyelitis Vaccine, Measles Vaccine, Live; Mumps Vaccine, Live; and Rubella Virus Vaccine, Live, has maintained a colony of South American monkeys, namely, marmosets (Saguinus mystax). Recently, interest in this primate has increased greatly since it has been found to be susceptible to Rous sarcoma virus and has been thought possible to be of use in the study of the pathogenesis of infectious hepatitis. Therefore, in October, 1967, the Animal Test Section obtained and established a colony of 86 marmosets. At present, there are 53 animals remaining. The problems of colony maintenance and nutrition are under control. Attempts to produce live young from the colony are being intensified in the hope that a nucleus of laboratory bred marmosets will be available for further studies. A complete microbiological profile, as well as certain baseline values, has been established. Studies concerning the usefulness of this marmoset as an animal model for human hepatitis are just underway.

The laboratory staff participate in a large program of collaborative research projects which are underway not only with other laboratories in the Division but with NCI, NIAID, NINDS, and NIMH. Some of these projects are specifically related to control activities of the Division and others have a tangential relationship. This collaborative research program is under the direction, supervision, and coordination of the Chief of the Laboratory.

Further, the Chief of the Laboratory has directed most of her own research efforts in the general field of the pathogenesis of infectious diseases with particular emphasis on oncogenic viruses. The pathogenesis of the particular tumors induced, the effect of altered immunologic mechanisms, and the interaction of these viruses on each other are all being studied. The inhibitory effect of one microbial agent on another both in regard to oncogenicity as well as infectivity is of particular interest. Thus, the inhibitory effect of adeno-associated viruses on the oncogenicity of adenovirus 12 in newborn hamsters is matched by the inhibitory effect of adenovirus 5 on the replication of certain viruses in hamster cell cultures.

In addition, two members of the staff of the Controls Operations office are engaged in research projects under the supervision of and in collaboration with the Chief of the Laboratory. One, Dr. Stanley Singer, is studying the basic interaction between microbial agents. He has found, as mentioned above, that certain viruses, such as adenovirus 5, inhibit the replication of other viruses, whereas, certain mycoplasmas enhance the replication of viruses. The other, Dr. John Petricciani, has just begun a study of the effect of in utero infection on chromosomes. Mr. Edward Fitzgerald, previously performing some studies under the direction of the Chief, Laboratory of Pathology, has transferred to the Laboratory of Control Activities in order to relate his studies more closely to certain control tests.

Dr. Amos E. Palmer of the Laboratory of Pathology was Project Officer for a contract set up to study the possible oncogenesis of biological products in newborn hamsters. Since Dr. Palmer's departure, Dr. Stanley Singer, Mr. Edward Fitzgerald, and Mrs. Claire Cox have monitored this contract closely. The activities involving this contract have been closely supervised by the Chief of the Laboratory of Pathology who is reviewing the histologic material evolving from this contract.

Since many of the contracts which are underway have aspects relating to pathogenesis, the Chief of the Laboratory of Pathology works very closely with the Contracts Operations Staff and spends much time helping to plan and execute and to review progress reports of those contracts already in existence. The Laboratory is cooperating in performing the pathologic examinations that are necessary for certain contract operations in which the contractor cannot do these examinations himself, namely, the contract on the effects of storage conditions on plastic blood containers. Histologic material from other contracts are under review particularly those involving the oil adjuvants.

LABORATORY OF VIRAL IMMUNOLOGY (LVI)

The program of the Laboratory of Viral Immunology can be described under three headings: (1) research, (2) control testing and (3) advisory support. Research represents the major responsibility of the Laboratory in terms of man-hours of effort. About two-thirds of the work could be described as research or research-associated. Control testing involves the assay of licensed or experimental biologics products provided by commercial concerns and the standardization of reference materials distributed for use in biologics control. The staff of the Laboratory supports the Office of the

Director in an advisory capacity in such matters as licensing of products, research contracts operations, investigational new drug applications and investigations of reports of possible adverse effects related to the use of biologics.

The research program is far-ranging; the only common denominator is the relation of the information sought to current or potential needs in the biologics field. Activities in this area will be highlighted in the following paragraphs:

Rubella. This program begun by the Laboratory's staff in 1963 should in the coming months provide a maximum return on invested effort. Two research achievements by our group, the attenuation of the virus in 1965 and the development of the HI test for immunity in 1966, served as a catalyst to final vaccine development. Rubella vaccine is expected to become commercially available before the end of fiscal year 1969. The current rubella research of the LVI is described in some detail in the individual project reports. Particularly important have been: laboratory and clinical characterization of a number of attenuated strains including each of the vaccines being considered for license; vaccine studies involving women (rubella-like reactions to the attenuated strains occur uniquely in adult females); and development of a laboratory model for the study of vaccine-induced immunity and resistance to reinfection.

In the area of control testing a rubella reference serum and a reference virus have been prepared. The serum has been standardized and distributed. The virus preparation is currently under test. Techniques being used by the potential manufacturers for production, potency assay and safety testing are being evaluated. In this way the reliability of their procedures will be ascertained well in advance of license.

We expect that the smooth transition from the experimental phase to license and general clinical use of vaccine will at least in part be due to our program begun six years ago in anticipation of future need.

Hepatitis. The control of hepatitis remains as one of the major challenges in virology. Its importance in the biologics field is two-fold: (1) some forms of hepatitis are transmitted by biologics products (blood and blood derivatives) and (2) if a vaccine were developed it might be possible to prevent hepatitis.

The causative virus or viruses of hepatitis have long eluded scientists. Over the years one or another false leads have produced brief optimism followed by disappointment. Because of the importance of the problem the LVI has maintained a small program of exploratory research. During the past year promising preliminary results have come from this effort. It appears that a virus-like material suspected of being the etiologic agent can be demonstrated by an immunologic technique and by electromicroscopic examination. If these particles are hepatitis virus then the availability of assay methods should speed research. For example, the methods would be of considerable assistance in the search for a method of culturing the virus.

Smallpox Vaccine. Responsible groups are increasingly concerned about the background of morbidity and mortality associated with the use of smallpox vaccine in relation to the decreasing risk of importation of the disease. Our program has anticipated this trend and for several years been committed to the study of variants of vaccinia virus and their possible value for immunization. Progress in this work is described in the individual project reports.

Measles and Mumps Vaccine. Major research efforts of the past were devoted to the study of these two vaccines as experimental products. Now in routine commercial use and recommended for administration to every American child these vaccines present continuing control obligations to the Division. During the past year perfection and application of a sensitive microtiter assay system has remarkably improved and simplified our potency testing of the products. This and similar test development projects are an important part of the Laboratory's research program.

Herpes-like Viruses. A great deal of attention has centered around the herpes-like virus, generally termed Epstein-Barr (EB) virus discovered several years ago in cultured cells derived from Burkitt lymphoma. The Section on Viral Genetics of the LVI has concentrated all of its effort on the study of the virus and its possible role in infectious mononucleosis and certain malignancies. Of immediate interest to the Division has been the principal investigator's demonstration of the association of EB virus presumably contained in transfused blood with the post-perfusion syndrome, an infectious nononucleosis type of illness.

Hereditable Diseases. As the study methods of modern medicine have become more sophisticated, a striking array of hereditable diseases have been recognized. Generally the traits involved are recessive and mothers may given birth to normal children, diseased children (homozygous) or carriers of the trait (heterozygous). The Cell Metablism Unit of the Laboratory is exclusively concerned with the application of cell culture techniques to genetic studies. This work has been in part of a fundamental nature seeking to chart the nature of the biochemical defect determined by the genetic flaw. However, a particularly exciting outgrowth of the basic research has been use of the techniques to establish an intra gestational diagnosis. Here amniotic cells aspirated in early pregnancy are cultured and studied to determine if the unborn infant will or will not have the disease. These results when positive can be used as a basis for the medical,interruption of pregnancy.

Cytomegalovirus. These highly species specific viruses are found in many animals as well as man. Simiam cytomegaloviruses are common contaminants of monkey kidney cell cultures. Because of the use of monkey kidney cultures for vaccine manufacture it is important to acquire information that could be used to detect potentially contaminated animals. The Laboratory has found that the epidemiology of simian cytomegalovirus resembles that of SV_{40}, another common virus of monkeys encountered in early work with poliovirus vaccines. Few monkeys harbor the virus in the jungle thus the rapid transport and quarantine methods developed to import monkeys free of SV_{40} and measles should furnish animals free of cytomegaloviruses.

Influenza Vaccine. A major portion of the Laboratory is devoted to the execution of the Division's responsibility with regard to influenza vaccine. A constant effort is required: (1) to maintain a competent Respirovirus Unit for the study of influenza viruses and other viral agents etiologically associated with respiratory tract infections of man; (2) to maintain a reference collection of infectious viral antigens and antibody reagents of influenza and other respiroviruses including those of potential importance as vaccine constituents; (3) to review and analyze manufacturers' protocols and to perform potency tests on current influenza vaccines; and (4) to improve serologic techniques for immunogenic potency testing of influenza vaccines.

Heretofore, the CCA test was considered unreliable as a measure of the antigen content of influenza vaccines because the test was not standardized. During this past year, great strides have been made to make the CCA test reproducible not only within a laboratory but also between different laboratories. This was especially helpful to use in carrying out our control mission during this season's epidemic caused by the A2/Hong Kong/68 variant.

DBS was able to make some of its vaccine (ordinarily used for the purposes of control) available to competent investigators early enough so that major volunteer studies were initiated. Our Laboratory was directly involved in 2 different studies which were completed prior to the major epidemic wave.

Yellow Fever Vaccine. The DBS yellow fever project was established in April 1966 as a coordinated research program involving several laboratories in the Division. Scientific profits have been realized from this team approach to problem solving.

As avian leukosis virus (ALV)-free primary yellow fever vaccine strain has been developed by ridding the 17D virus vaccine of its ALV contaminant. By differential filtration through Millipore membrane filters of different pore size, the smaller yellow fever virus was separated from its larger contaminant by physical means. The ALV-free candidate primary and secondary seed lots were indistinguishable from corresponding ALV-contaminated lots with respect to: (1) potency as measured by titration in newborn and weanling mice and in the MA-104 plaque system; (2) degrees of viscerotropism as measured by viremia in monkeys; (3) neurotropism as determined by the monkey neurovirulence test; and (4) potency as determined by antibody response in monkeys inoculated by the intracerebral route.

Studies on immunogenicity were conducted on a total of 265 rhesus monkeys and on 180 volunteers at the Lewisburg Federal Penitentiary. The ALV-free yellow fever vaccine was indistinguishable from the currently available commercial ALV-contaminated vaccine with respect to reactions and to antibody response.

In the course of these studies a plaque neutralization test was developed; it has many practical and theoretical advantages over the standard yellow fever mouse neutralization test.

Smallpox Vaccine. A system of enzyme treatment for smallpox vaccines has been devised which reduces the extraneous debris sufficiently that a direct electron microscope particle count of the virus can be made. The direct count of virus particles provides a quick, easy quantitation which can then be used to correlate other methods of titration. Studies on aggregation-dispersion performed with smallpox vaccine virus, passed once in cell culture to eliminate calf lymph debris, indicate that aggregation increases the infective titer.

Adenovirus Vaccine. At the present time adenovirus vaccines are not being released for use in the United States, and, hence, are not generally available to the public. Surprising as it may seem, little is known about the biology of human adenovirus infection. The Laboratory continues its research studies on adenoviruses with the hope that, eventually, the proper place for adenovirus vaccines in the armanmentarium of respirovirus vaccines will be known.

In searching for an experimental animal our Laboratory was unsuccessful in attempts to infect rhesus monkeys with human adenovirus type 4. A fortuitious accident and keen investigative studies established the fact that human adenovirus type 31 caused an outbreak of coryza among chimpanzees.

Egg Vaccines. The developing chicken embryo remains one of the most versatile substrates for vaccine manufacture. In order to maintain vaccine purity, it has been necessary to survey avian flocks for potentially troublesome microbial infections and to maintain chicken flocks which are free of known viral, bacterial and mycoplasma contaminants. The Avian Leukosis and Immunofluorescence Units of this Laboratory provide the DBS with the competency to deal with potential problems in this area.

Studies on the group specific antibody of avian leukosis viruses in experimentally infected pigeons might simplify the testing for these endogenous chicken viruses. This would be advantageous both to vaccine manufacturers and DBS.

Studies have demonstrated the usefulness of substituting egg yolk antibody for serum antibody in the immunofluorescence test to study agents of all microbial groups (viruses, rickettsiae, bedsoniae, mycoplasmas, and bacterial. The capability of the immunofluorescence test to detect avian leukosis viruses from the different subgroups makes it an important adjunct to the RIP test in the safety testing of human viral vaccines of egg origin.

Studies are being performed to determine whether Marek's disease of chickens can be transmitted via the egg. The extent and significance of this potential contaminant will be evaluated with the hope of developing satisfactory methods for testing potential contamination in chicken embryos to be used for vaccine production.

Rickettsial Vaccines. As with most killed antigens, it is difficult to measure the potency of rickettsial vaccines. Emphasis was directed toward evaluating a candidate lot of inactivated typhus vaccine for use as a reference antigen. Although this reference vaccine evoked a good antibody response in man, it was considered sub-optimal as a reference on the basis of its direct complement-fixing antigen titer and marginally satisfactory with regard to its guinea pig potency titer. Future studies will be concerned with evaluating

what correlation exists between the laboratory tests for potency and the immunological response evoked in man.

Additional studies concerned: (1) definition of the nutritional requirements of the intracellular parasite Rickettsia quintana, and (2) comparison of the genetic relatedness of rickettsial and other intracellular parasites.

Arbovirus Studies. With abolishment of NIAID's Laboratory of Tropical Virology, the Arbovirus Unit of our Laboratory became the only facility on the NIH reservation with the professional and technical competency capable of performing studies on arboviruses. The working collection of viral and antibody reagents has expanded considerably. Present reagents representing a number of virus strains for most of the major and minor arbovirus groups are available and being utilized for routine and special studies.

A comparison of several methods for preparing arbovirus hemagglutination (HA) and complement-fixation (CF) antigens demonstrated that adequate HA antigens could be made simply by extraction of suckling mouse brains (SMB) suspensions with genetron and that satisfactory CF antigens could be made from 20X virus-infected cell concentrates, crude and genetron extracted SMB suspensions.

In addition, our laboratory (in collaboration with Biomedical Engineering and Instrumentation Branch, DRS) completed development of a negative pressure isolator modified for work with larger animals. Certain features of this isolator make it more versatile than previously described isolators made from flexible plastic. This isolator allowed us to isolate the virus of simian hemorrhagic fever with absolute safety to other monkeys. Experiments using potential human pathogens could be done with a similar degree of safety.

Hemorrhagic Fever Studies. Previous reports described this Laboratory's isolation of the heretofore unknown virus of simian hemorrhagic fever which was responsible for a devastating epizootic of hemorrhagic fever among colonies of rhesus monkeys at the Primate Quarantine Unit, LAB (Fall, 1964), and at the Sukhumi Institute of Experimental Pathology and Therapy, USSR (July 1964). We have shown that a subsequent epizootic at the National Center for Primate Biology, Davis, California (September 1967) was caused by the same virus. Recent reports suggest that a similar disease occurred in a monkey colony in Great Britain. The significance of knowing more about potential hazardous diseases of monkeys is obvious.

Studies were also performed on 2 members of the Tacaribe group of American hemorrhagic fever viruses. Adult guinea pigs immunized with live Amapari virus did not resist challenge with Junin virus (Argentinian hemorrhagic fever virus) to which they are normally quite susceptible. This was quite unexpected because, as reported earlier, immunization with Tacaribe virus (another member of the same virus group) did protect guinea pigs from subsequent challenge with Junin virus.

Immunobiological Studies. Of major concern in studies of delayed hypersensitivity has been the development of an in vitro test, particularly for man, and the discovery of the factor or factors that confer a passive hypersentisitivity

state. Previous studies using human RNA species in vitro have shown the transfer of an immunological message to previously nonsensitive human lymphoid cells. Defining the mechanism of activity is paramount for evaluating RNA as a biologic product.

Other studies were performed to evaluate the relative importance of cellular and humoral factors in determining individual responses to revaccination. Following revaccination, volunteers with major skin reactions to vaccinia demonstrated: (1) significant increases in lymphocyte thymidine incorporation compared to pre-immunization levels of reactivity, and (2) the appearance of antigen specific migration-inhibition-factor (MIF).

Studies directed toward the isolation and immunology characterization of cell antigens revealed that antigens with strikingly different immunochemical patterns could be isolated from cell extracts. Additional studies are required to determine the significance of these findings. Histocompatibility antigens and cell-associated antigens obtained in a water soluble state offer many advantages over whole cells in regard to antigenic identification. This work shows promise in such areas as: (1) identification of culture variability and antigenicity; (2) control and standardization of transplantation immunology; (3) selectivity and potency evaluation of anti-lymphocyte globulin; (4) tumor cell identification and standardization; and (5) a better understanding of autoimmune disorders.

Viral Pathogenesis Studies. A new class of unusual agents found to cause diseases in man (kuru, subacute sclerosing panencephalitis) and animals (scrapie, visna, maedi) show unusual properties, such as long latent incubation period of months to years, protracted disease course, and pathologic changes unlike those of acute viral diseases. Studies with this new class of infectious agents and possible contaminants of biological products will provide DBS with the competency to develop testing and monitoring techniques.

Additional studies were performed on autoimmune reactions related to viral and adjuvant sensitization and on improving techniques for isolation and study of viral agents affecting the central nervous system.

Cell Culture Studies. A program of research and development in connection with production of cell cultures for the Division is actively pursued. Studies involved: (1) standardization of techniques applicable to large-scale production of cell cultures; (2) development of new cell culture systems as possible substrates for vaccine production and testing; (3) determining the viability of trypsinized tissues at 4C and the use of serum protein fractions in cell culture system; and (4) identification of specific in vitro cell differential antigens.

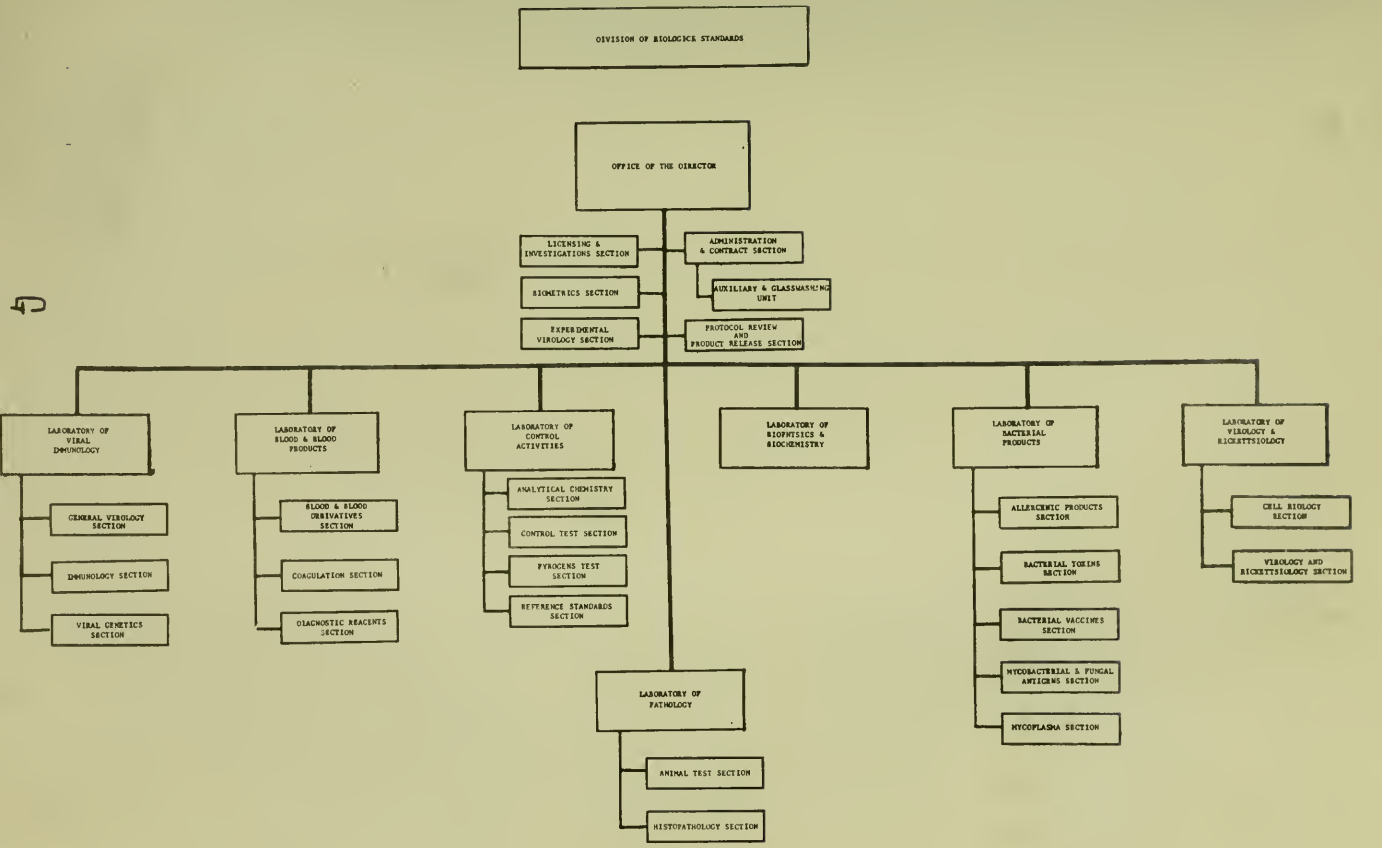
As a result of these studies, many practical improvements have been introduced into routine cultivation of cells. Increasing emphasis is being given to studying the general biology and immunobiology of cultured cells.

Cell Cultures. The Cell Biology Section continues to spend most of its effort on the purely service function of providing the entire Division with quality cell cultures for research and control uses. Much effort has been made to improve the Section's service functions and to insure adequate cell production. This service results in a considerable saving to the Division. All of the DBS continuous cell lines have been replaced with certified, Mycoplasma-free cells and the cell repository is operating at an efficiency which should allow almost immediate return to full scale production in the event of accident or contamination.

Avian Leukosis Testing. The Avian Leukosis and Immunofluorescence Units are responsible for testing vaccines for potential contamination with viruses of the avian leukosis complex. In addition they routinely check egg derived vaccines for the presence of other avian contaminants (e.g. reticuloendotheliosis (RE) virus). During this reporting period a total of 251 RIF and FAB (fluorescent antibody) tests were performed, of which 9 were on 8 live measles vaccines, 2 on lots of experimental rubella vaccines, 6 on 5 rubella vaccines submitted in support of license application, 3 on lots of yellow fever vaccines, and 21 were tests on an experimental measles vaccine. All these tests were negative.

This Laboratory is also responsible for monitoring the NIH RIF-free chicken flock. There were 210 tests performed on eggs from SPAFAS and the NIH flocks, egg yolks and chick embryo cell cultures.

Influenza Vaccines. With the onset of this season's influenza epidemic caused by the A2/Hong Kong/68, LVR's Respirovirus Unit and the Division's Laboratory of Control Activities mobilized their forces: (1) to obtain and distribute to the manufacturers a virus strain suitable for vaccine manufacture; (2) to prepare, standardize, and distribute a reference vaccine (which is being regarded as one of the best reference influenza vaccines); and (3) to streamline channels of communication so that vaccine lots could be released, at times, within 24 hours of receipt of a satisfactory protocol from the manufacturers. Because of our successful efforts to make the CCA test simple and reproducible, potency testing of the monovalent vaccine was not the chore it used to be. In addition, reference vaccines of civilian (69C) and military (69M) formulation have been prepared for distribution to the manufacturers for the forthcoming 1969-70 season. The rapidity with which these reference vaccines were prepared and certified by testing attests to the very practical benefit derived from this year's control-related research efforts.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ANIMAL TEST SECTION
HISTOPATHOLOGY SECTION

Annual Report of the
Laboratory of Bacterial Products
Division of Biologics Standards
July 1, 1968 through June 30, 1969

There was one change in the research staff of the Laboratory. Dr. Vito M. Esposito transferred to LBBP, DBS on March 1. The enthusiasm and motivation of the staff to add to the knowledge in their respective fields, especially as it relates to high quality of bacterial and allergenic products, have continued at a high level. The staff keeps abreast of the newest knowledge applicable to old line products and to other products on clinical investigations for which application of license may be filed and in turn standards will need to be formulated. The programs of the experienced staff would benefit if new positions were available.

Besides their active research programs, the staff actively participates in the regulatory functions of the Division, in cooperative intramural, national and international projects, in collaborative (contract) programs of the Division and other Institutes and in professional and educational activities of scientific societies. The special competence of the staff is recognized by invitations to lecture, serve on scientific committees, study groups, etc.

1. Research Activities

Tuberculins. The polysaccharide fraction isolated from tuberculin which was as active as the protein fraction PPD in detecting tuberculin sensitivity of guinea pigs was also as effective as PPD in inhibiting migration of cells of sensitive guinea pigs, but not in the induction of blast cell transformation in sensitized cells and only 20 percent of tuberculosis patients gave positive skin reactions. The patient activity, however, was specific, nontuberculosis patients did not react. Protein fractions remain the diagnostic dermal agent of choice (Proj. No. 100, p.10).

Studies directed towards differentiation of tuberculosis from "atypical" mycobacterial sensitivity by means of diagnostic dermal substances have shown (1) a better understanding of the antigenic relationship between the sensitizing species of mycobacteria, (2) a correlation of *in vitro* lymphocyte transformation with delayed human skin reactivity to test antigens including histoplasmin and (3) a difference in dermal reactivity of PPD and OT licensed products with equal tuberculin unitage in guinea pigs sensitized to several atypical mycobacterial species, respectively. (Proj. No. 135, p.43 and 103, p. 39). The consensus of investigators, the Tuberculosis Section, NCDC and the Diagnostic Committee, American Thoracic Society, is that the best differential diagnostic dermal test would be the simultaneous use of 5 TU of tuberculin and Battey antigen and that each new batch of tuberculin be characterized for human reactivity in four designated areas of the United States. Implementation will be a long term project. The 1968 Tuberculin Conference provided valuable information and stimulus towards the tuberculin projects.

Tuberculosis Vaccines. A study was initiated to characterize 16 strains of BCG used in different countries for the production of BCG vaccine. The

cultures, received through the courtesy of the Trudeau Institute, are showing some marked differences. The study is directed towards determining optimum characteristics of strains for BCG vaccine. (Proj. No. 101, p. 38.)

Poison Ivy and Related Extracts. The elucidation of the nature of the chemical structure of the active principle of *Rhus* plants as delayed contact sensitizers and whether or not desensitization can be effected has been a much more complex problem than anticipated. However, there has been a steady progression of publications of applicable basic information from Contract PH-43-64-76 and LBP investigators. One significant finding this year is that poison oak urushiol has a 17 carbon side-chain and poison ivy urushiol has a 15 carbon side-chain. Currently pure urushiol supplied by Dr. Dawson is under clinical investigation (Ph-43-68-632) to determine if sensitive persons can be desensitized. Preliminary results are encouraging. It is anticipated that this study will establish or refute the claims that poison oak extract is capable of desensitization of man. *Rhus* plants cause the most frequent and incapacitating contact sensitization illness in the United States. Additional information has been obtained by LBP workers on the dependence of the specificity of the delayed reaction to catechols as well as the immune tolerance on size and shape of the side-chain of the molecule, on the absorption of PDC and PDV on protein and their distribution to all internal organs, and on the effect of a series of substituted halodinitrobenzenes on delayed hypersensitization. (Proj. No. 102, p. 12.)

Standardization of Allergenic Extracts. Studies have shown that skin reactivity of sensitive persons to short ragweed pollen extracts correlates with the Antigen E content of the extract, determined by the gel-diffusion method, and with the histamine release from the white cells of the patients. (Proj. No. 119, p. 13.) Significant differences were observed between lots of extracts. There was no correlation with PNU content. Attempts to develop a practical assay for Antigen E in ragweed pollen extracts are in progress and the laboratory and clinical study is being extended to other pollen extracts (NIH-69-2010). Confirmation of the correlation of laboratory assayed content of pollen extracts with their clinical specificity would provide a breakthrough in the development of specific requirements for the potency of these extracts. Other findings are that storage of short ragweed pollen for 20 years did not markedly alter the quality of the extracts, and that different extracting fluids do not give equivalent extracts (PH-43-68-1250). Consistent with the concept that mites in house dust are the cause of allergic activity of the dust, mites have been found in samples of house dust used for preparation of extracts (NIH-69-65). Mites isolated and cultured will be used to further the study on house dust extracts.

Mycoplasma. (Proj. No. 17, p. 49) Besides contributing a possible harmful effect to vaccines prepared from microorganisms grown in tissue cell culture or chick embryo, mycoplasmata may cause direct effect on the metabolism of the tissue cells by depletion of an essential amino acid such as arginine as reported last year. This year in collaboration with LCA it was shown that two species of *Mycoplasma* promote the yield of a virus by causing a decrease in amount of interferon normally induced by the virus. Factors affecting photoinactivation of *Mycoplasma* have been determined. A new species, *M. arginini*,

was isolated from tissue of several animal species and characterized.

From three of the DBS contracts, new information has been acquired on the mycoplasmal flora of several species of animal used as source of primary cell cultures for propagation of viruses for vaccines as well as on cultural procedures for the isolation and detection of these elusive organisms in those vaccines which are subject to mycoplasma-contamination risk. Knowing what species to search for and how to detect them during the manufacture of a vaccine provides greater assurance of the safety of the product.

Tetanus Studies. The final phase of the collaborative study on the prevention of neonatal tetanus in New Guinea (Proj. No. 12, p. 18) showed that after two primary injections of adsorbed tetanus toxoid a mean protective titer (0.01 u/ml) was provided for 40 months but not for 54 months. Earlier observations showed that three injections of plain toxoid provided a protective titer for only six months. Antitoxin levels of women injected with one dose of oil emulsified toxoid also, declined after 40 months. A higher antitoxin response was seen in women with abscess at site of injection than in those without abscess. Prebooster antitoxin titers determined booster response which was around 10 fold. Again, superiority of adsorbed over plain toxoid for primary immunization is emphasized. Mean antitoxin response to booster adsorbed toxoid was only 1.7 greater than to plain toxoid; no information was obtained on relative duration of response. If confirmed that there is a direct relation between assayed unitage of plain and adsorbed toxoids and the duration of human antitoxin levels, a requirement for quantitative unit assay for tetanus toxoids would be indicated (Proj. No. 14, p. 22).

The study (Proj. No. 13, p. 20) on the separation and characterization of antigens in filtrates of *Clostridium tetani* showed the presence of two different esterases, the activities of which were enhanced by human and rabbit gamma globulin and not associated with an immune phenomenon. Antihemolysin was induced in rabbits and monkeys by injection of crude and commercial tetanus toxoids. Activity of certain components of toxin appear to be related to symptoms of tetanus in man other than spastic paralysis. Results may be applicable to standards for Tetanus Toxoid Immune Serum Globulin (Human).

Water-in-oil Adjuvants. In the study to determine factors responsible for sterile abscess formation in recipients of oil emulsified biologics, the use of tissue culture cells continued to show promise. Four of 19 lots of Arlacel A were toxic for cells and three of the four were toxic by the mouse assays; 6 were non toxic by both tests. There was a direct correlation between FFA content and cell toxicity. The cell-culture test will be used to study toxicity of various diluents and chemically defined components of oil adjuvants that have been separated and studied for activity by other methods (DBS contracts).

Typhoid Vaccine. (Proj. No. 116, p. 32.) In the study for the development of a potency assay that would reflect the efficacy of various types of typhoid vaccine, focus was placed on the different types of immunological response of three strains of mice to Vi antigen as a component of whole cell vaccine, and Vi fraction in solution or absorbed to erythrocytes. Vi hemagglutinin, bacterial agglutinin and active cutaneous anaphylaxis responses differed with

reference to the physical state of the Vi antigen and between the mouse strains. There was no correlation between these immunological responses and the protective response of mice. Although the protective response (ED_{50}) differed between mouse strains, two gave the same relative potencies between antigens. Another strain gave a significantly larger difference. This could explain differences in relative potencies of vaccines obtained between laboratories. Vi antigen isolated by a new procedure was significantly more protective for mice than Vi antigen prepared by a published method.

Pertussis Vaccine. (Proj. No. 104, p. 30.) The research program on pertussis vaccine was continued towards elucidation of the reactive factors of *Bordetella pertussis*, and means for laboratory detection and assay of these factors in vaccines, and the relation of the factors to untoward reactions in vaccinated children. Special emphasis was placed on the newly described lymphocyte-promoting factor. The laboratory testing phase of the DBS-PMA cooperative study has been completed and results are ready for statistical analysis. The clinical phase is yet to be initiated. It has been shown with use of the *Rhesus* monkey, as others have shown with use of other animal species, that pertussis vaccine accelerates the onset of experimental allergic encephalomyelitis. No relation of this reaction to the so-called pertussis vaccine encephalopathy has been established.

Cholera Vaccine and Toxins. (Proj. No. 22, p. 26.) This study is closely tied in with the vaccine field trials and bacteriological studies of the Pakistan-SEATO Cholera Research Laboratory, Dacca, East Pakistan and with cholera investigations of other workers in order to obtain basic information applicable to composition and control testing of protective cholera antigens. The Fy 69 field trials showed that monovalent Ogawa vaccine did not protect against Inaba infection whereas the Inaba vaccine afforded significant protection. The results confirm this Laboratory's potency data of the vaccines.

One of the several investigators on the separation of the cholera toxin from somatic antigens has developed what appears to be a breakthrough method that will permit large volume production of toxin for preparation of a toxoid for field study not later than 1971. This Laboratory is currently investigating the separated toxin for the presence of somatic antigens, methods of detoxification, immunological response of animals to toxin and toxoid and an in vitro method for titration of antitoxin response that would be practical for the field study.

A correlation between vibriocidal titers and resistance of man to cholera has been established by PSCRL. However, vibriocidal antibodies are found in persons outside an endemic cholera area. A cross antigenic relation observed by Gallut between somatic antigens of *Brucella* and *V. cholerae* has been confirmed. In addition, it was shown that sera of convalescent brucellosis patients have not only high and long-lasting vibriocidal titers against *V. cholerae*, particularly the Inaba type, but pronounced passive protective activity against mice challenged with *V. cholerae* (with Dr. Gangarosa).

Vibrio cholerae gastrointestinal infection of mice. (Proj. No. 136, p. 35.) With the view that gastrointestinal flora may be responsible for

variable natural resistance of man to *V. cholerae*, the role of indigenous microflora on resistance of mice to this infection is being studied. *V. cholerae* was established (without cholera symptoms) in the GI tract of germ-free and gnotobiotic mice infected with either of two lactobacilli, one enterococcus and one bacteriodes, singly or in combination, and housed in an isolator but not when housed in a barrier room environment. Attempts are being made to find the presence of other bacteria such as the fusiformers as the cause of resistance of the latter mice.

Laboratory test animals. The influence of the animal species, the difference between strains of a species and the intestinal microbial flora of animals on the nature of the immunological and toxic response in the evaluation of biological products does not have widespread recognition. Growing out of the significant differences in the response of mouse strains to pertussis vaccine, a cooperative project with DRS of selectively breeding out of the NIH strain a strain with uniform desirable responses to pertussis vaccine was initiated and is progressing satisfactorily (Proj. No. 138, p. 36). Proj. No. 116, p.33 emphasized the importance of the mouse strain in determination of the potency of typhoid vaccine in relation to human efficacy.

With the increasing production of microbially controlled mice, it is important to determine the effect of the intestinal flora in control testing. Previous reports showed that germ-free and specific pathogen free mice were so much more resistant to pertussis vaccine than conventional mice that they could not be used for toxicity testing. The effect of microbial control mice on potency assay of vaccines is under investigation (Proj. No. 136, p. 34).

Basic information about the immunological response of the marmoset was obtained in collaboration with LP (Proj. No. 159, p. 17). The small size of this primate makes it attractive as a test animal for biologics but there is little information about its immunological response as compared with the monkey species commonly used.

2. Control Activities

A. Regulatory Standards

a. Assisted technically in matters pertaining to the publication of anthrax and typhoid vaccines and tuberculin standards.

b. Technical document pertaining to standards for BCG vaccine is in preparation.

B. Potency and Safety Tests (January 1, 1968 through December 31, 1968)

The Laboratory of Bacterial Products performed tests on and/or reviewed protocols for 9 products as follows:

Tests and protocol reviews

Type of product	Purpose	Type of test: Potency	Type of test: Safety Toxicity	Protocols reviewed
Pertussis Vaccine	Official*	104	76	51
	Special, foreign	7	13	
	Standardization	29		
Cholera Vaccine	Coop. LCA			21
	Special	12	4	
Pertussis Immune Globulin (Human)	Official	8		4
Haemophilus influenzae typing serum	Official			4
BCG Vaccine	Official	23**		23
			Mycoplasma only	
Measles Vaccine	Official		9	122
Mumps Vaccine	Official		1	7
Poliovirus Vaccine	Official			6
Rubella Vaccine	Official		1	
Intramural			62	
Total		183	166	238

*Tests of samples of lots for release, inspection or license application.
**Viability counts.

C. Activities requiring travel status.

Type of activity	Personnel	Locations	Man days of travel
Annual inspections	9	31	47
Contracts	3	21	29

D. License applications and amendments and notices for investigation of new drugs (IND)

Category	Number	Person participation
License	35	96
Labeling	40	68
IND	30	44

E. Other Activities.

The Chief, LBP. Project Officer for the Pakistan-SEATO Cholera Research Laboratory (PL-480 Project #08-002-1 FIC)

PSCRL, Member of Panel of Expert Consultants to the Technical Committee

NIH Cholera Advisory Committee, Member

Committee on Immunization, Armed Forces Epidemiological Board, Associate Member

U. S. Pharmocopeia, Member of Advisory Panel for Sterility Testing and Sterilization

Guest Lecturer, Howard University

Board of Governors, American Academy of Microbiology and Chairman, Exhibit Committee

Archives Committee Member, American Society for Microbiologists

WHO Temporary Advisor, Biological Standardization, January 18-25, 1969

NIAID Special Program, Ad Hoc Pneumococcus Vaccine Committee, Member

Dr. Harold Baer. Committee on Standardization of Allergens, American College of Allergy

Committee on Specific Therapy, American Academy of Allergy

Education Committee, American Association of Immunologists

Dr. S. D. Chaparas. Faculty Chairman, Microbiology and Immunology, NIH Graduate School

Dr. John C. Feeley. (see Honors and Awards, Proj. No. 22, p. 29) Cholera Panel, U.S.-Japan Cooperative Medical Science Program

Advisory Editor - Journal of Infectious Diseases

WHO Member of Expert Advisory Panel on Bacterial Diseases (Cholera), 1967-72

Project Officer for three NIAID contracts pertaining to cholera toxins

Staff members have audited conferences, workshops and given lectures.

3. Collaborative (Contract) Activities

One staff member served as Project Officer for six contracts that are an extension of the research program of the Allergenic Products Section, and another member served as Assistant Project Officer on five of these contracts. Three contracts relate to *Rhus* extracts: the isolation and synthesis of the active principles of poison ivy, poison oak and poison sumac and preparation of components for laboratory and clinical investigations (PH-43-64-76 p.256), and clinical desensitization by use of urushiols, the active principles of *Rhus* extracts (PH-43-64-885 p.258and PH-43-68-632 p.281). Contract PH-43-64-885 terminated this year. The other three contracts involve the chemical properties of pollens and pollen extracts (PH-43-68-1250 p.284), the isolation identification and cultivation of mites from house dust (NIH-69-65 p.285) and the human potency of allergenic extracts of grass and ragweed pollen extracts (NIH-69-2010 p.289).

Three members are serving as Assistant Project Officers on nine other DBS contracts and one member is serving as Project Officer for three NIAID contracts on cholera toxin (PH-43-67-1428, PH-43-67-1369 and PH-43-68-673).

Serial No. DBS 100
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Composition, Immunology, and Specificity of Tuberculin

Previous Serial Number: SAME

Principal Investigators: Harold Baer, Ph. D.
S. D. Chaparas, Ph. D.
H. P. Godfrey, M. D.

Other Investigators: Sheldon M. Wolff, M. D. (NIAID)
Richard K. Root, M. D. (NIAID)
Joseph Gallelli, Ph. D. (CC)

Cooperating Units: Laboratory of Clinical Investigation, NIAID
Pharmaceutical Development Service, CC

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: (1) To determine whether the polysaccharide that can elicit a delayed sensitivity reaction in the skin of guinea pigs sensitized with BCG can inhibit the migration of peritoneal exudate cells. (2) To determine whether the polysaccharide can elicit skin reactions in tuberculin positive humans. (3) To study dialyzable tuberculin.

Methods Employed: (1) The migration of peritoneal exudate cells was examined by the method of George & Vaughn and David. The cells from sensitive and non-sensitive animals are placed in capillaries and incubated in a tissue culture medium in the presence and absence of carbohydrate. (2) Patients with and without tuberculosis were tested intradermally with polysaccharide and PPD solutions. (3) Dialyzable tuberculin is being fractionated into components of varying molecular size.

Major Findings: (1) The polysaccharide was as effective as PPD in inhibiting migration of cells. It did not induce blast cell transformation in sensitized cells (Godfrey, Baer, and Chaparas). (2) No tuberculin positive patient without tuberculosis reacted to the polysaccharide but about 20% of

tuberculosis patients did react (Baer, Wolff, Root, and Chaparas). The polysaccharide was shown previously to react in almost all sensitive guinea pigs.

Significance to Bio-Medical Research and the Program of the Division: This is the first demonstration that a polysaccharide can function in the inhibition of cell migration system. The fact that it reacts in the skin of only a few tuberculin positive individuals suggests that its skin reactivity is unrelated to any tuberculin protein that it might contain.

Proposed Course of Project: Studies will be continued on the dialyzable tuberculin.

Honors and Awards: None

Publications:

Godfrey, H. P., Baer, H., and Chaparas, S. D.: Inhibition of Macrophage Migration by a Skin Reactive Polysaccharide from BCG Culture Filtrates. J. Immunol. (In press).

Serial No. DBS 102
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Immunology of the Delayed Contact Sensitivity Induced by Catechols Related to and Contained in Poison Ivy Extracts

Previous Serial Number: SAME

Principal Investigators: Harold Baer, Ph. D.
Henry Godfrey, M. D.

Other Investigators: C. J. Maloney, Ph. D. (OD)

Cooperating Units: Radiation Safety Department (CC)
Office of the Director

Man Years

Total:	2.5
Professional:	1.25
Other:	1.25

Project Description:

Objectives: (1) To examine the composition and to establish a method for the standardization of poison ivy extracts and related plants such as poison oak and sumac. (2) To determine the relationship of structure of catechols to their effectiveness in inducing delayed contact sensitivity and immune tolerance, and (3) to study the mechanism of delayed contact sensitivity.

Methods Employed: (1) Guinea pigs, sensitized to various synthetic catechols, were used to evaluate the relationship of catechol structure to immunologic specificity. (2) Urushiol, extracted from poison ivy leaves by Professor Dawson, was catalytically reduced in the presence of tritium to yield radioactive pentadecylcatechol (PDC). This material was used to trace the distribution of PDC in guinea pigs after topical application. Tritiation of dimethylurushiol yielded radioactive pentadecylveratrol (PDV), which was used to trace the distribution of nonsensitizing material similar to PDC after topical application. (3) Studies were carried out with guinea pigs sensitized to a series of substituted halodinitrobenzenes as models of delayed hypersensitivity.

Major Findings: (1) Specificity of the delayed reaction to catechols as well as immune tolerance to catechols depends on the size and shape of the side chain. (2) PDC and PDV are rapidly absorbed into the blood after application to intact skin and are distributed to all internal organs. High concentrations of metabolites rapidly appear in urine, bile and feces. (3) The degree of contact sensitivity induced by the halodinitrobenzenes appears to be negatively correlated with atomic volume of the halogen substituent while the amounts of serum antibodies induced correlate with ability of the small molecule to react with protein.

Significance to Bio-Medical Research and the Program of the Division: These studies are providing information on the mechanism of sensitization by catechols and on the relationship of chemical structure to ability to induce delayed contact sensitivity. The results of the studies with halodinitrobenzenes have provided insight into the mechanisms underlying delayed hypersensitivity and have served as a useful comparison to the studies with catechols.

Proposed Course of Project: The project will be continued until all major objectives have been reached.

Honors and Awards: None

Publications:

Baer, H., Dawson, C. R., and Kurtz, S. P.: Delayed Contact Sensitivity to Catechols. IV. Stereo chemical conformation of the Antigenic Determinant. J. Immunol. 104:1243-1247, Dec. 1968.

Serial No. DBS 119
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Standardization of Allergenic Extracts

Previous Serial Number: SAME

Principal Investigators: Harold Baer, Ph. D.
Henry Godfrey, M. D.

Other Investigators: Donald E. Hill (OD)
Philip Norman, M. D., Johns Hopkins
Lawrence Lichtenstein, M. D., Johns Hopkins

Cooperating Units: Office of the Director
Johns Hopkins Medical School

Man Years

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description:

Objectives: (1) To measure the presence, stability, and quantity of Antigen E in commercial ragweed extracts. (2) To immunize rabbits with extracts of various pollens to produce antisera capable of estimating the quantity of extract in mixed commercial extracts. (3) To study extracts of pollens from various crop years to determine whether the antigen content is affected by crop year or by storage of pollen.

Methods Employed: (1) Gel diffusion to establish the presence of Antigen E in ragweed extracts and to estimate the quantity present. (2) Skin tests in humans and histamine release studies to determine whether the Antigen E content can be correlated with dermal activity in humans. (3) Preparation of extracts from pollens of at least 8 different crops that have been stored up to 20 years. The extracts will be studied qualitatively and quantitatively for antigen content.

Major Findings: (1) The skin activity of sensitive humans of short ragweed extracts correlates with the Antigen E content of the extract and with the release of histamine from the white cells of the patient. (2) Storage of short ragweed pollen up to 20 years does not markedly alter the quality of extracts obtained from it.

Significance to Bio-Medical Research and the Program of the Division: The correlation of the Antigen E content with the *in vivo* activity of extracts of short ragweed pollen suggests that it may be possible to specify the potency of licensed ragweed allergenic extracts by measuring their content of Antigen E and that the method may be applicable to other pollen extracts.

Since pollen can be stored for extended periods of time and the variation in crop year is relatively small it means that extracts low in potency were probably the result of either mishandled pollen or improperly repared extracts.

Proposed Course of Project: (1) To determine whether a practical assay for Antigen E can be devised. (2) To extend the studies to other pollens and their extracts.

Honors and Awards: None

Publications: None

Serial No. DBS 134
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Standardization of Methods of Skin Testing Humans for Allergy

Previous Serial Number: SAME

Principal Investigators: Harold Baer, Ph. D.
William B. Grater, M. D.

Other Investigators: B. A. Berman, M. D.
R. Hale, M. D.
M. Scherr, M. D.
W. Swinney, M. D.
C. J. Maloney, Ph. D. (OD)

Cooperating Units: Office of the Director
Committee on Standardization of the American College of Allergy

Man Years

Total:	0.1
Professional:	0.05
Other:	0.05

Project Description:

Objective: It was the purpose of the Committee to attempt to determine whether stringently controlled conditions could improve quantitation of skin testing of allergic patients.

Methods Employed: Ragweed extract was injected at varying dilutions into the skin of sensitive individuals; simultaneously, a single dose of histamine was injected as a control.

Major Findings: The data have not as yet been analyzed.

Significance to Bio-Medical Research and the Program of the Division: Individuals differ somewhat in their response to histamine. Since the response to ragweed extract is largely the result of liberation of histamine, it may be possible to evaluate better the response to allergenic extracts if the histamine response is known.

Proposed Course of Project: This will be determined by the outcome of the current project.

Honors and Awards: None

Publications: None

Serial No. DBS 159
1. Bacterial Products
2. Allergenic Products
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Immunology of the Marmoset, Saguinus mystax

Previous Serial Number: None

Principal Investigators: Harold Baer, Ph. D.
Douglas Lorenz, Ph. D. (LP)

Cooperating Unit: Laboratory of Pathology

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objectives: To study the delayed sensitivity and antibody response of marmosets.

Methods Employed: One group of marmosets was injected with fluorodinitrobenzene (DNFB) in complete Freund's adjuvant containing heat-killed BCG while in a second group dinitrophenyl bovine serum albumin (DNP-BSA) was substituted for the DNFB. At various intervals, the animals were tested intradermally with tuberculin, PPD and cutaneously with DNFB. The animals were also bled to test for antibody.

Major Findings: (1) Only a few marmosets developed delayed sensitivity to DNFB in contrast to Hartley strain guinea pigs in whom almost all develop sensitivity. (2) Almost all of the marmosets became tuberculin positive. However, the tuberculin reaction was large, erythematous, somewhat diffuse and was not exactly like tuberculin reactions in either the guinea pig or human. (3) Only about 10% developed antibody to DNP-BSA, none to BSA.

Significance to Bio-Medical Research and the Program of the Division: (1) Marmosets may become useful as primate test animals for biologics because of their small size. It is useful, therefore, to know how their immunological responses resemble or differ from other animals. (2) If tuberculosis develops in these animals during quarantine or long term holding it could be detected by routine tuberculin testing.

Honors and Awards: None

Publications: None

Serial No. DBS 12
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigation of Tetanus Toxoids for Prevention of Neonatal Tetanus

Previous Serial Number: SAME

Principal Investigators: Margaret Pittman, Ph. D.
Michael F. Barile, Ph. D.
M. Carolyn Hardegree, M. D.

Other Investigators: Frank Schofield, M. D., New Guinea

Cooperating Unit: Department of Public Health, Territory of Papua and New Guinea

Man Years

Total:	0.7
Professional:	0.5
Other:	0.2

Project Description:

Objectives: (1) To determine the persistence of tetanus antitoxin in New Guinea women immunized since 1962 with adjuvant and plain toxoids. (2) To evaluate the booster response in these women after a plain or an adsorbed toxoid.

Methods Employed: The mouse neutralization test was used to determine antitoxin levels of the sera of the women.

Major Findings: (1) The antitoxin titers after primary immunization with plain toxoids were maintained above the protective level (0.01 u/ml) for only 6 months whereas the titers after primary immunization with adjuvant toxoids were maintained for 40 months. The antitoxin response was not influenced by age whereas a higher response was seen in women with abscess at site of injection of oil emulsified toxoids than in those women without abscess. (2) In the booster series the lowest post booster titers were found in women primarily immunized with plain toxoids. Pre-booster titer determined the booster response which was around 10 fold. The mean antitoxin response in women receiving boosters with $AlPO_4$ adsorbed toxoid was 1.7 times greater than the mean antitoxin response in women receiving a booster of plain toxoid. Seven percent

(7%) of the women did not respond to primary immunization with the $AlPO_4$ toxoids.

Significance to Bio-Medical Research and the Program of the Division: Information has been obtained on the duration of the antitoxin level which protects against neonatal tetanus as influenced by adjuvants. The field results provide information on human antitoxin response to the laboratory assayed potency of tetanus toxoids. The findings are applicable to the potency and safety specifications for tetanus toxoid.

Proposed Course of Project: (1) To publish the laboratory and clinical findings. (2) to continue studies to determine cause of untoward reactivity of oil adjuvants (Proj. No. 15).

Honors and Awards: None

Publications: None

Serial No. DBS 13
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: A Study of the Antigenic Substances of *Clostridium tetani*

Previous Serial Number: SAME

Principal Investigator: M. Carolyn Hardegree, M. D.

Other Investigator: Mr. Harry Marshall (LBB)

Cooperating Unit: Laboratory of Biophysics and Biochemistry

Man years

Total:	0.75
Professional:	0.25
Other:	0.5

Project Description:

Objectives: (1) To determine and separate the antigens present in culture filtrates of *Cl. tetani*. (2) To characterize the *in vivo* and *in vitro* activities of these substances. (3) To determine their relationship to optimal anti-tetanus immunization.

Methods Employed: Column chromatography, zone electrophoresis, gel diffusion, immunoelectrophoresis, enzyme assays, plasma hemoglobin determination, thin layer chromatography, gas chromatography, animal bioassay, animal immunization.

Major Findings: (1) Two esterases, separated by column chromatography, were distinct from the neurotoxin, the hemolysin and the gelatinase. The activity of both esterases was enhanced by human and rabbit gamma globulin; this is not an immune phenomenon. (2) Using gas chromatography it was demonstrated that the esterases in crude culture filtrate are not substrate specific for a single fatty acid ester in the Arlacel A. (3) Antihemolysin was produced in rabbits and monkeys after immunization with crude and commercial tetanus toxoids.

Significance to Bio-Medical Research and the Program of the Division: It is expected that the investigation will contribute to an understanding of the role of antibodies against *Cl. tetani* antigens other than the neurotoxin.

These antibodies may be necessary for the prophylaxis and the treatment of tetanus. Findings will provide fundamental information for the control of tetanus toxoid and tetanus immune serum globulin.

Proposed Course of Project: Studies will be continued on (1) the isolation and recovery of components of the culture filtrates, (2) the continued characterization of the *in vivo* and *in vitro* effects of the hemolysin, (3) the relation of the esterases to adverse reactions of antigens in oil adjuvants and the nature of the effect of globulin on the esterases. Studies will be initiated to evaluate the effects of *Cl. tetani* filtrates on blood coagulation and on the sympathetic nervous system.

Honors and Awards: None

Publications: None

Serial No. DBS 14
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Standardization of Reference Preparations for Tetanus Toxoids

Previous Serial Number: SAME

Principal Investigators: Margaret Pittman, Ph. D.
Michael F. Barile, Ph. D.
M. Carolyn Hardegree, M. D.
Robert W. Kolb, M.S. (LCA)
Edward B. Seligmann Jr., Ph. D. (LCA)

Other Investigators: None

Cooperating Unit: Laboratory of Control Activities

Man Years

Total:	0
Professional:	0
Other:	0

Project Description:

Objectives: (1) To establish a unitage for a U. S. reference toxoid. (2) To correlate laboratory assayed potency with the antitoxin response of women in New Guinea. (3) To relate these findings to the U. S. potency requirement for tetanus toxoid.

Methods Employed: Mice and guinea pigs were vaccinated with graded doses of tetanus toxoids and their responses were determined (1) by challenge with tetanus toxin and (2) by titration of antitoxin levels of guinea pig sera by the mouse neutralization test.

Major Findings: Findings are being prepared for publication.

Significance to Bio-Medical Research and the Program of the Division: The international reference tetanus toxoid adsorbed provides a basis for comparison of assayed potency of adsorbed tetanus toxoids prepared in all nations. (An international reference for the plain toxoids was designed earlier.) It will be possible to assay the unitage of U. S. tetanus toxoid

by use of the correlated U. S. references and to relate laboratory assayed unitage to antitoxin response of New Guinea women.

Proposed Course of Project: The findings will be prepared for publication. Attempts will be made to determine the feasibility of applying the information to the U. S. requirements for potency of tetanus toxoids. This project will be terminated.

Honors and Awards: None

Publications: None

Serial No. DBS 15
1. Bacterial Products
2. Bacterial Toxins
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigation of Toxicity of Oil Emulsified Antigens

Previous Serial Number: SAME

Principal Investigators: M. Carolyn Hardegree, M. D.
Michael F. Barile, Ph. D.

Other Investigators: Ruth Kirschstein, M. D. (LP)

Cooperating Unit: Laboratory of Pathology

Man Years

Total:	1.7
Professional:	0.85
Other:	0.85

Project Description: Part I. The toxicity of oil adjuvant components and free fatty acids in animals.

Objectives: To determine factors responsible for sterile abscesses in recipients of oil emulsified biologics.

Methods Employed: (1) Free fatty acids of various oil adjuvant components were determined. (2) Mice, rabbits and guinea pigs were injected intraperitoneally, intramuscularly or subcutaneously with components of oil adjuvants and with free fatty acids. The gross and microscopic pathology was observed and the weight change of mice was determined.

Major Findings: (1) Free fatty acids (FFA) in quantities detected in emulsified antigens associated with sterile abscesses did not prevent weight gain in mice or induce granulomas in rabbits. (2) Arlacel A containing comparable levels of FFA did induce granulomas in rabbits. (3) These data suggest that FFA per se are not responsible for sterile abscesses. (4) Three of 19 lots of Arlacel A were found to be toxic by the mouse assay. A correlation was seen between the amount of FFA and the toxicity in the mouse assay.

Project Description: Part II. The toxicity of oil adjuvant components in cell culture.

Objectives: To examine the use of cell cultures for the evaluation of oil adjuvant component toxicity and to evaluate the mechanism of this toxicity.

Methods Employed: (1) Cultures of 6 cell lines were inoculated with various oils and emulsifiers and the cell toxicity was measured. (2) The effect of various diluents on the toxicity of the oils and emulsifiers was determined. (3) A comparison was made between the *in vitro* and *in vivo* toxicity.

Major Findings: (1) A cell culture assay was developed to evaluate the toxicity of Arlacel A. (2) Four of 19 lots tested were found to be toxic in cell cultures. Eighteen of 19 lots gave similar results in the cell culture and mouse assays. (3) A correlation was seen between the FFA content and the toxicity of Arlacel A. (4) A characteristic cytotoxic effect consisting of intracytoplasmic refractile bodies was produced by the toxic lots.

Significance to Bio-Medical Research and the Program of the Division: These studies provide information on the reactivity of components of oil emulsified antigens and may be useful in developing specifications for oil emulsified products should the use of oil adjuvants be approved.

Proposed Course of Project: To continue to evaluate various *in vivo* procedures for the evaluation of the toxicity of the components of oil adjuvants and to continue to evaluate the relationship of the FFA in the emulsifier, Arlacel A, to reactivity. To use cell cultures to evaluate the toxicity of other adjuvant components. To prepare findings for publication.

Honors and Awards: None

Publications:

Hardegree, M. Carolyn and Kirschstein, Ruth: The Toxicity of Free Fatty Acids and Arlacel A. *Annals of Allergy* 26: 259-268, May 1968. (Reported as "In Press" in 1968.)

Serial No. DBS 22
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on Cholera and Cholera Vaccine

Previous Serial Number: SAME

Principal Investigator: John C. Feeley, Ph. D.

Other Investigators: Margaret Pittman, Ph. D.
Vito M. Esposito, Ph. D.
Carl E. Miller, D. V. M.
H. Donald Hochstein (Guest Worker)
Joseph W. West (LCA)

Cooperating Units: Laboratory of Control Activities
U.S.-Japan Cooperative Medical Science Program
Pakistan-SEATO Cholera Research Laboratory
(NIH Research Agreement 196802)
Johns Hopkins University School of Medicine

Man Years

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description:

Objectives: (1) Establishment of a meaningful standard of potency for cholera vaccine; (2) Investigation of significance of cholera toxin antigen as an immunizing agent; (3) Examination of the antigenic relationship between Brucella and Vibrio cholerae; (4) Subspecies characterization of strains of V. cholerae from recent outbreaks of cholera.

Methods Employed: Quantitative potency assays of vaccine were performed in mice. Sera were obtained from rabbits immunized with experimental vaccines, toxins and toxoids and analyzed by agglutination, vibriocidal, hemagglutination, gel diffusion and skin-toxin (permeability factor) neutralization tests. Routine bacteriological and serological methods were employed in examination of cultures.

Major Findings: Subproject 1: Standardization of cholera vaccine. In the Pakistan-SEATO Research Laboratory 1968-69 field trials in which freeze-dried monovalent Ogawa and Inaba vaccines were employed, the Ogawa vaccine provided little or no protection against cholera caused by Inaba serotype

whereas the Inaba vaccine provided substantial protection. We found that the mouse protection assays of the vaccines correlated well with the field trial results in that the Ogawa vaccine was only 0.03 times as protective as Inaba vaccine against Inaba challenge. Inaba vaccine gave a higher degree of protection against Ogawa challenge but this observation could not be related to human efficacy due to lack of Ogawa infections.

Subproject 2: Cholera Toxin Studies. The enhanced antigenicity of formalinized crude cholera toxin for guinea pigs reported last year has been shown to be true also for rabbits. These findings are in press.

Preliminary acrylamide gel immunoelectrophoretic studies on crude preparations have shown that formalinized cholera toxoid antigen has greater electrophoretic mobility than the toxin antigen. Whether this is due to increased electrical charge or possible polymerization to the basic toxin molecule by formalin awaits further investigation.

In the study of the highly purified cholera toxin prepared by Dr. S. H. Richardson of Bowman-Gray School of Medicine, which contains a single protein staining band in disc electrophoresis, we confirmed the toxic activity by immunoelectrophoresis and skin testing. A single band was produced in immunodiffusion plates against crude cholera antitoxin. This purified toxin produced no significant febrile response or observable reactions when one million bluing doses were injected intramuscularly or intravenously in rabbits. Immunologic studies to assess the agglutinating, vibriocidal and toxin neutralizing antibody response of these rabbits to single and booster doses of toxin are currently in progress.

Preliminary findings show promise that passive hemagglutination tests with erythrocytes sensitized even with crude concentrated cholera toxin can be developed for the assay of cholera antitoxin titers.

Preliminary findings indicate that some separation of somatic antigens from toxin activity may be achieved by the use of exclusion chromatography on porous glass columns. (Collaborative study with LCA)

Subproject 3: Antigenic relationship between Brucella and Vibrio cholerae. Agglutinin and agglutinin absorption tests on rabbit sera have confirmed the observations of Gallut that the antigenic cross reactivity between these species is due to a heat-stable somatic antigen that is more dominant in the Inaba serotype than the Ogawa serotype of V. cholerae. This phenomenon has been ascribed often to a heat-labile flagellar antigen of V. cholerae.

In collaborative studies with Dr. E. J. Gangarosa of the National Communicable Disease Center, rabbits immunized with brucella and convalescent brucellosis patients have been found to have very high and long-persisting vibriocidal antibody levels against Vibrio cholerae, particularly the Inaba serotype. These sera have pronounced protective activity in passive mouse protection tests.

Subproject 4: Characterization of strains of Vibrio cholerae. All of fourteen strains isolated during an outbreak in Chittagong, East Pakistan in 1968 were of the Ogawa serotype and belonged to type 4 in our previously described scheme. According to current terminology, these strains are weakly hemolytic "El Tor" vibrios. This outbreak is the first substantial occurrence of "El Tor" instead of "classical" cholera vibrios in East Pakistan. An interesting feature of this outbreak is that 5 of these freshly isolated strains had the characteristics of "rough" strains (i.e. agglutination in acriflavine and anti-rough serum). Rough strains are generally considered avirulent.

Significance to Bio-Medical Research and the Program of the Division: Establishment of a correlation of laboratory potency data with efficacy in man as determined in controlled field trials is essential for establishment of a meaningful standard of potency for cholera vaccine. The results of the field trial with monovalent cholera vaccines are in essential agreement with laboratory potency data in that Ogawa vaccine showed very little protection against cholera due to Inaba serotype. This is also a clear indication that a monovalent vaccine produced from the Ogawa serotype would be unacceptable for general use. Comparable information is needed from field trials on cross-protection between serotypes when the infecting organism is of the Ogawa type.

In view of evidence that presently available vaccines which stimulate antibacterial immunity cannot be expected to yield a high degree of long-term protection, especially in the high-risk young age group, there is great interest in development of immunizing agents which induce antitoxic immunity. Studies such as those in which we are involved are providing basic information of importance in the development and standardization of an appropriate toxin or toxoid type antigenic preparation for eventual controlled field trial in man.

Studies on antigenic relationship between cholera vibrios and brucella are of epidemiologic interest insofar as they may have bearing on naturally occurring immunity to cholera. Patients infected with brucella develop very high and persistent vibriocidal antibody titers, and the presence of "cholera" vibriocidal antibodies is well correlated with immunity in susceptible populations.

The continued monitoring of characteristics of strains of Vibrio cholerae from different epidemics is being carried out in relation to any possible implications for changes in the strain composition of cholera vaccine. Present facts do not suggest the need for any such change, although recommendations for inclusion of "El Tor" strains in the vaccine have been made by some workers.

The apparent establishment for the first time of a focus of "El Tor" infection in East Pakistan may have some implications for future field trials should the study area become involved. In India, "El Tor" strains have all but displaced "classical" strains with concurrent changes in the epidemiologic picture, especially in relation to a lower incidence of clinically severe cholera.

Proposed Course of the Project: Studies will be continued along lines outlined above.

Honors and Awards:

Dr. J. C. Feeley
(1) Member, NIH Cholera Advisory Committee 1968.
(2) Member, WHO Scientific Group on Cholera Immunology (Teheran, Sept. 14-19, 1968).
(3) Adjunct Associate Professor of Parasitology at the School of Public Health, University of North Carolina.

Publications:

Carpenter, C.C.J., Sack. R. B., Feeley, J. C. and Steenberg, R. W.: Site and characteristics of electrolyte loss and effect of intraluminal glucose in experimental canine cholera. J. Clin. Invest. 47:1210-1220, 1968 (reported last year as "in press")

Feeley, J. C.: Research on cholera: A renewed attack on an ancient scourge. Texas Rep. Biol. Med. (in press).

Feeley, J. C. and Roberts, C. O.: Immunological responses of laboratory animals to cholera vaccines, toxin, and toxoid. Texas Rep. Biol. Med. (in press).

Serial No. DBS 104
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on Pertussis Vaccine

Previous Serial Number: SAME

Principal Investigators: Margaret Pittman, Ph. D.
Charles R. Manclark, Ph. D.
Ronald G. Clark, Ph. D. (LP)

Other Investigators: Ruth L. Kirschstein, M. D. (LP)
Vernon Wong, M. D. (NINDB)
James F. Marshall

Cooperating Units: Laboratory of Pathology
Ophthamology Branch, NINDB
Pharmaceutical Manufacturers' Association

Man Years

Total:	2.6
Professional:	1.0
Other:	1.6

Project Description:

Objectives: The overall objective is that the pertussis vaccine component of products issued by U. S. licensed establishments has optimum properties: adequate and stable potency through the dating period and relative freedom from untoward reactivity. Objectives of current studies are (1) to relate laboratory assayed reactivity of vaccines and fractions of the constituent bacteria, as determined by multiple tests, to clinical reactivity; (2) to find the cause of the neurological reactions that on rare occasions follow pertussis vaccination, and to develop a laboratory assay that measures the reactive factor, should it be identified.

Methods Employed: Mouse potency tests to determine adequacy and stability of potency. Analysis of results at regular intervals to determine any reflected trend. Measurement of pertussis vaccine toxicity (reactogenicity) by determination of the early and late weight changes in mice, the lethal dose in mice, histamine sensitizing activity, necrotizing activity in the skin of suckling mice, capacity to induce edema in foot pad of the rat, rabbit pyrogenicity, ability to promote lymphocytosis and/or leucocytosis in the mouse, etc. Induction of toxic and immunologic encephalopathies in monkeys, rats, mice, and

guinea pigs. Electroencephalograms, retinal and optic tract examinations, blood and spinal fluid chemistry of monkeys, and histopathology of all animals.

The laboratory assayed reactivities of two well-characterized pertussis vaccines are currently being determined in cooperation with manufacturers of pertussis vaccine and will be related to reactivity in children. The clinical study is under the supervision of Dr. Harry M. Meyer (LVI) and a representative of the PMA.

Major Findings: Subproject 1: No significant changes in potency and toxicity of pertussis vaccines submitted for release were noted. Studies on the characterization and isolation of lymphocyte promoting factor from pertussis vaccine are still in the preliminary phase.

Subproject 2: The collaborative DBS-PMA laboratory assays of the study vaccines have been completed and the results will be sent to the statisticians for study and analysis.

Subproject 3: Adolescent and newborn Rhesus monkeys manifest encephalopathies when sensitized with complex mixtures of homologous (Rhesus) or heterologous (guinea pig) spinal cord, Freund's complete adjuvant, and pertussis vaccine. Rats (Fischer 344/N) were also reactive but the reaction rates and symptoms were significantly less than that for the monkey. Monkeys which received pertussis in addition to Freund's adjuvant and heterologous spinal cord showed clinical symptoms in one-half the time required for those which received guinea pig spinal cord and Freund's adjuvant alone. The major clinical signs in the monkey were ataxia, motor weakness and paralysis with optic tract envolvement in a significant number of animals. Histopathological examination revealed typical lesions of allergic encephalitis in varying stages of development. Some animals showed central nervous system lesions in the absence of overt clinical symptoms. Lesions were confined primarily to the brain stem and involved basal ganglia, optic system, and corpus callosum. Relatively few lesions were found in forebrain structures and none were observed in spinal cord. These studies are continuing.

Significance to Bio-Medical Research and the Program of the Division: The study is related directly to the control of the potency and safety of pertussis vaccine and the relation of laboratory assayed toxicity to untoward reactivity in children. Encephalopathy that follows vaccination, in rare instances, is a distressing reaction and the experimental studies are directed toward the elucidation of the cause. This project also provides basic immunological information on Bordetella pertussis.

Proposed Course of Project: To continue the project as outlined above.

Honors and Awards: None

Publications:

Pittman, M.: Bordetella pertussis - bacterial and host factors in the pathogenesis and prevention of whooping cough. In Mudd, S. (Ed.): Infectious Agents and Host Reactions, Philadelphia, Pa., W. B. Saunders Company, 1969, Chapter 11. (In press)

Serial No. DBS 116
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on Typhoid Vaccine

Previous Serial Number: SAME

Principal Investigator: Vito M. Esposito, Ph. D.

Other Investigators: John C. Feeley, Ph. D.
Margaret Pittman, Ph. D.

Cooperating Units: None

Man Years

Total:	1.3
Professional:	0.7
Other:	0.6

Project Description:

Objectives: The overall objective was to develop a potency assay that reflects efficacy of all types of typhoid vaccines for man. Initial objectives were to determine: (1) the mechanism(s) of protection in the mouse evoked by immunization with typhoid vaccines, and (2) the role of constituent antigens in these processes.

Methods Employed: Routine serological and bacteriological procedures, mouse protection tests and various physicochemical techniques were employed.

Major Findings: The Vi-antibody, active cutaneous anaphylaxis and protective responses (ED_{50}) of three mouse strains (CFW, NIH and Balb/cAnN) to acetone-inactivated typhoid vaccine and soluble Vi antigen showed that after immunization with vaccine, mice of each strain had similar Vi-hemagglutinin titers, whereas after immunization with soluble Vi antigen, only NIH and Balb/cAnN had Vi hemagglutinins. Bacterial agglutinins were detected only in CFW and NIH mice injected with vaccine. Significantly better protection against challenge was obtained with each mouse strain by the intraperitoneal (i.p.) than by the subcutaneous (s.c.) route of immunization. The latter confirms previous findings of this and other laboratories. Significant differences in protective responses (ED_{50}) were observed between the three mouse strains vaccinated with either Vi antigen or typhoid vaccine. NIH mice immunized with

vaccine or Vi antigen exhibited active cutaneous anaphylaxis when challenged with vaccine or Vi antigen. CFW mice likewise immunized did not develop active cutaneous anaphylaxis. The serological responses of the strains of mice to Vi antigen did not correlate with their protective responses.

In rabbits, Vi antigen administered in particulate form induced hemagglutinins, bacterial agglutinins, and passive cutaneous anaphylaxis (PCA) antibodies, whereas soluble Vi antigen induced only hemagglutinins. Guinea pigs passively sensitized with antisera against particulate, but not soluble, Vi antigen gave PCA reactions when challenged with either soluble or cellular Vi antigen. The specificity of PCA was demonstrated by its dependence on the Vi concentration and by absorption of PCA activity from antisera with V-form cells of Salmonella typhosa. The results indicated that the physical state of Vi antigen is important in the type of immune response induced.

The plaque forming ability of spleen cells from mice immunized with typhoid vaccines and purified Vi antigen was tested against viable S. typhosa cells and Vi coated erythrocytes. S. typhosa organisms devoid of Vi antigen were more susceptible to the bactericidal action of immune spleen cells. Vi containing S. typhosa organisms were resistant to killing by immune spleen cells in the presence of complement even when the cells were producing Vi antibody.

A new method was developed for the isolation of Vi antigen, and this led to the isolation of two additional antigenic components (antigens 1 and 2). This Vi antigen preparation was a significantly better protective antigen for mice than the Vi antigen used in earlier studies. NIH mice were better protected by all three antigens than were CFW mice. In the protection test, Vi and antigen 2 were more protective by i.p. immunization, whereas antigen 1 was more effective by the s.c. route. These results provide additional evidence of the variable response of mouse strains to Vi antigen and emphasized the differences in response to s.c. and i.p. routes of immunization.

Significance to Bio-Medical Research and the Program of the Division: In earlier studies it was shown that the U. S. prescribed potency assay reflected human efficacy for acetone and heat-phenolized typhoid vaccines but not for alcoholized vaccines which are presumably high in Vi antigen content. Determination of the influence of the various antigens, particularly Vi antigen, and the influence of the route of vaccination on the immunological response of mice are of importance to provide assurances that potency test methods reflect human efficacy.

Proposed Course of Project: The principal investigator transferred to LBBP on March 1, 1969. The project will be resumed when the position is filled. Three manuscripts have been submitted for publication and a fourth manuscript is in preparation for publication.

Honors and Awards: None

Publications: None

Serial No. DBS 136
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Evaluation of Microbially Controlled Animals in Control Testing and Infection

Previous Serial Number: SAME

Principal Investigators: Carl E. Miller, D.V.M.
John C. Feeley, Ph. D.

Cooperating Unit: None

Man Years

Total:	1.0
Professional:	0.6
Other:	0.4

Project Description: Part I. Microbially Controlled Animals in Control Testing.

Objectives: To determine the effects of utilization of microbially controlled (i.e. so-called "caesarean-derived barrier sustained" or "specific pathogen-free") animals having a greatly reduced variety of microbial flora on control tests of bacterial vaccines.

Methods Employed: Simultaneous tests for potency and freedom-from-toxicity with various vaccines (e.g. pertussis, typhoid, cholera) are being performed in parallel using "conventional" and microbially controlled mice of the same strain.

Major Findings: Part I. Due to a continuing shortage of required numbers of microbially controlled mice, no additional tests comparing results with conventional mice could be performed.

Project Description: Part II. Role of the Indigenous Microflora in Resistance of Mice to Infection with Vibrio cholerae.

Objectives: To determine which microorganism(s) are responsible for natural resistance to gastrointestinal infection with Vibrio cholerae in mice.

Methods Employed: Plastic isolators are used to control the microbial environment of V. cholerae infected mice. These mice are inoculated with pure

cultures of various intestinal bacteria from conventional mice to assess the role of the normal flora in competing with V. cholerae. In some experiments, cholera-mono-contaminated mice are removed from the isolator, handled with sterile gloves, given an oral dose of the competing bacteria and placed in sterile filter-bonnet cages. The mice are cultured periodically for 1-2 weeks and necropsied at the termination of the test. Any indication of successful competition in these animals will be repeated in the germfree isolator where all microbial factors can be controlled.

Major Findings: Vibrio cholerae was established in the GI tract of a gnotobiotic mouse housed in an isolator and infected with two lactobacilli, an enterococcus, and a strain of bacteroides, singly or in combination. However, microbially controlled mice from a barrier room environment whose bacterial flora contained the above organisms were not infected with V. cholerae. Currently, the possible occurrence of other organisms such as certain non-cultivatable, fusiform-like bacteria is being investigated. Experiments are in progress to assess the role of these fastidious organisms in cholera infected mice.

Significance to Bio-Medical Research and Program of the Division: In the past, the quality of laboratory animals has been controlled largely by their physical environment, nutrition, and genetics. Now the biological environment is being controlled and this has a pronounced effect on the health and response of the animal to stress. This is evident from our previous observation that a pertussis vaccine could be considered non-toxic in the quality controlled animals and still have significant toxicity based on tests with conventional animals. This has many important implications in control testing of biologic products.

The question of why only some persons in a given environment develop cholera is not completely explained on the basis of immunologic consideration. Other factors, such as the intestinal flora, may play a significant role in competing with the cholera organism. Identification of the factor(s) which permit or prevent colonization in mice may help explain the absence of cholera in exposed individuals.

Proposed Course of Project: Continuation along the lines outlined above.

Honors and Awards: None

Publications: None

Serial No. DBS 138
1. Bacterial Products
2. Bacterial Vaccines
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Selective Breeding to Establish a "Standard" Mouse

Previous Serial Number: SAME

Principal Investigators: Margaret Pittman, Ph. D.
Carl T. Hansen, Ph. D. (DRS)
Charles R. Manclark, Ph. D.

Other Investigators: James F. Marshall
William J. Hinkle (DRS)

Cooperating Unit: Division of Research Services

Man Years

Total:	0.6
Professional:	0.2
Other:	0.4

Project Description:

Objectives: To establish a mouse strain that has superior and consistent characters for the potency and toxicity assays of biologics, particularly bacterial vaccines.

Methods Employed: Forty or more pairs of the NIH mouse strain have been selected. The progeny of each are being tested for ability to be sensitized to histamine by pertussis vaccine. Mice with the highest ability are being selected for further breeding. It is anticipated that another year or two will be required to establish the colony.

Major Findings: (1) Mice can be selected and bred for a genetically determined propensity to be sensitized to histamine following injection with pertussis vaccine. (2) Mice in the second and successive generations of selective breeding have demonstrated a significant increase in their ability to be sensitized to histamine following injection with pertussis vaccine.

Significance to Bio-Medical Research and the Program of the Division: The availability of a "standard" mouse for potency and toxicity of pertussis vaccine and other bacterial products should decrease variability in tests and give greater assurance of the validity of the estimate of the potency and toxicity of the products.

Proposed Course of Project: (1) To continue the study as described above. (2) To study the nature and inheritance of the genetic markers which determine the ability of a mouse to be sensitized to histamine. (3) To pursue basic immunogenic studies directed toward the establishment of an isogenic strain of mouse which carries the genetic marker(s) for histamine sensitivity and reactivity to pertussis protective antigen(s).

Honors and Awards: None

Publications: None

Serial No. DBS 101
1. Bacterial Products
2. Mycobacterial & Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on Tuberculosis Vaccine

Previous Serial Number: SAME

Principal Investigator: Sotiros D. Chaparas, Ph. D.

Other Investigator: Sally R. Hedrick

Cooperating Units: None

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objective: Characterization of BCG vaccines.

Methods Employed: Cultures of organisms representing BCG vaccines from sixteen different countries have been received from the Trudeau Institute. Studies are planned to investigate similarities and differences in the morphology, stability to freeze drying, immunogenicity, and antigenicity of these organisms.

Major Findings: Differences in texture and pigmentation of the colonies are noted. Four of the strains of BCG are able to reduce dye contained in the growth medium.

Significance to Bio-Medical Research and the Program of the Division: All the strains were originally derived from the Bacillus of Calmette and Guerin. The differences reflect the influence that conditions of cultivation have exerted on the original strain and emphasize that strains with different properties are being employed for production of BCG vaccines.

Proposed Course of Project: To continue and extend investigations. To characterize the various BCG strains for properties desirable in the selection of a reference or standard preparation.

Honors and Awards: None

Publications: None

Serial No. DBS 103
1. Bacterial Products
2. Mycobacterial & Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Hypersensitivity and Specificity of Fungal Diagnostic Substances for Dermal Tests

Previous Serial Number: SAME

Principle Investigators: Sotiros D. Chaparas, Ph. D.
Edward B. Seligmann, Ph. D. (LCA)
Vernon Fuller, Ph. D. (LCA)

Cooperating Unit: Laboratory of Control Activities

Man Years

Total:	0.3
Professional:	0.2
Other:	0.1

Project Description:

Objective: To develop suitable laboratory procedures for use in potency and identity tests for Fungal Diagnostic Substances for Dermal Tests.

Methods Employed: (1) The reagents being investigated are Coccidioidin, Histoplasmin and Blastomycin. Guinea pigs are subjected to different sensitization regimes of fungal antigen alone or in combination with spores or hyphae. These are emulsified in complete or incomplete Freund's adjuvant. Skin testing will be done on homologous and heterologous substances. (2) Lymphocyte transformation studies have been carried out with cells from sensitive human beings.

Major Findings: (1) Results are not yet available for the guinea pig potency and identity tests. (2) Peripheral leukocytes from individuals who were skin reactive to histoplasmin became transformed in vitro in the presence of histoplasmin. Although some histoplasmin positive individuals were negative to 5 units each of PPD and Battey antigen, their cells became transformed in the presence of these antigens.

Significance to Bio-Medical Research and the Program of the Division: Results of the study should provide information which is directly applicable to the development and improvement of the standards for fungal diagnostic substances for dermal tests.

Proposed Course of Project: Work will continue in efforts to develop satisfactory potency and identity tests for fungal antigens.

Honors and Awards: None

Publications:

Lunde, M., Chaparas, S. D., Diamond, L. Intradermal Reactions in Guinea Pigs to Entamoeba histolytica Antigen. Proc. Soc. Exp. Biol. Med. 130: 455-457, February 1969.

Serial No. DBS 120
1. Bacterial Products
2. Mycobacterial & Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Reports
July 1, 1968 through June 30, 1969

Project Title: Pathogenesis of Lesions Induced by Antigens in Water-in-Oil Emulsions

Previous Serial Number: SAME

Principal Investigator: Sotiros D. Chaparas, Ph. D.

Other Investigator: Ruth L. Kirschstein, M. D. (LP)

Cooperating Unit: Laboratory of Pathology

Man Years

Total:	0
Professional:	0
Other:	0

Project Description: This project has been terminated.

Honors and Awards: None

Publications:

Chaparas, S. D., and Kirschstein, R. L.: The role of delayed hypersensitivity and toxicity of BCG in the development of lesions at sensitization sites. Proc. Soc. Exp. Biol. Med. 129: 601-605, November 1968.

Serial No. DBS 135
1. Bacterial Products
2. Mycobacterial & Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Mycobacterial Antigens: Immunological Specificity and Antigenic Analysis

Previous Serial Number: SAME

Principal Investigator: Sotiros D. Chaparas, Ph. D.

Other Investigators: Sally R. Hedrick
John N. Sheagren, M. D. (NIAID)
Anthony DeMeo, M. D. (CC)
Robert Garman, D.V.M. (DRS)
Thomas Wera, M. D. (OD)
Edward B. Seligmann, Jr., Ph. D. (LCA)
Clifford J. Maloney, Ph. D. (OD)

Cooperating Units: Laboratory of Clinical Investigation, NIAID
Office of the Director
Clinical Center
Laboratory Aids Branch, DRS

Man Years

Total:	1.2
Professional:	0.6
Other:	0.6

Project Description: This project is divided into VI parts.

Objectives: The overall objectives are: (1) to determine the specificity, potency and identity of various mycobacterial antigens; (2) apply in vitro procedures of hypersensitivity for determining which organisms are responsible for human sensitivity to mycobacterial antigens; (3) determine reactivity of licensed tuberculins in guinea pigs sensitized with "atypical" mycobacteria; (4) detection of tuberculosis in monkeys by the in vitro stimulation of their leucocytes to tuberculin.

Part I. In vivo studies of the specificity of antigens prepared from various mycobacterial species. (In collaboration with Dr. Clifford J. Maloney.)

Methods Employed: Antigens from six species of Mycobacterium were skin tested in homologously and heterologously sensitized guinea pigs.

Major Findings: When all antigens were adjusted to a predetermined level of skin reactivity in homologously sensitized animals, the degree of reactivity of an antigen in its homologous system was not exceeded by any of the heterologous preparations. Differences in cross reactivities between the various antigens fitted a linear model which permitted a better understanding of the antigenic relationships. The U. S. Standards for Tuberculin, PPD and OT reacted differently in guinea pigs sensitized with each of three mycobacterial species.

Part II. Correlation of in vitro lymphocyte transformation with delayed skin reactivity. (In collaboration with Dr. John N. Sheagren and Dr. Anthony DeMeo).

Methods Employed: Guinea pigs and human beings were skin tested with various mycobacterial antigens. Lymphocytes from lymph nodes of sensitized guinea pigs and from peripheral blood of human beings were cultured in the presence of various antigens. The degree of lymphocyte transformation was determined by the degree of uptake of thymidine-H^3.

Major Findings: A correlation of delayed skin reactivity with in vitro lymphocyte transformation was found for guinea pigs and human beings.

Part III. Reactivity of licensed tuberculins in guinea pigs sensitized with "atypical" mycobacteria. (In collaboration with Dr. E. B. Seligmann.)

Methods Employed: Guinea pigs were sensitized with heat-killed M. intracellulare (Battey) and M. scrofulaceum (Gause). The reactivities of 12 licensed PPD and OT tuberculins which had been standardized in animals sensitized with M. tuberculosis were then compared to reactivity of the respective standard in the animals sensitized with the "atypical" mycobacteria.

Major Findings: Six of the tuberculins were more reactive than the respective standard in guinea pigs sensitized with Battey or Gause organisms.

Part IV: Correlation of in vitro lymphocyte transformation in monkeys with tuberculous infection. (In collaboration with Dr. Robert Garman.)

Methods Employed: Monkeys held in quarantine at NIH are skin tested for tuberculin reactivity. Positive and doubtful reactors are bled and their leucocytes are cultured in the presence of tuberculin. The degree of lymphocyte transformation is determined by the uptake of thymidine-H^3.

Major Findings: A correlation was found in that tuberculin positive monkeys were always tuberculous and their lymphocytes became transformed by tuberculins in vitro. Of the doubtful reactors, transformation occurred with only cells from those in which tuberculosis was confirmed post mortem.

Part V: Studies on Series I - Mycobacteria from International Working Group on Mycobacterial Taxonomy (IWGMT). (In collaboration with Dr. Thomas Wera.)

Methods Employed: Antigens have been prepared from 13 mycobacterial species. Reactivities are being studied by delayed skin tests and by lymphocyte transformation. Analyses of antigenic components are being made by acrylamide gel electrophoresis.

Major Findings: The greatest degree of skin test specificity is seen in guinea pigs sensitized with the homologous organisms. This specificity is also seen by in vitro transformation of lymphocytes. Acrylamide electrophoresis patterns are different for each of the antigen preparations studied thus far.

Part VI: Development of laboratory control identity and potency tests for mycobacterial diagnostic substances for dermal tests. (In collaboration with Dr. E. B. Seligmann.)

Methods Employed: M. intracellulare (Battey), M. scrofulaceum (Gause), M. fortuitum and M. kansasii (Bostrom) were obtained from the tuberculosis program of the National Communicable Disease Center. These organisms are being grown and will be freeze-dried for distribution to interested manufacturers. Interest is shown in Battey for antigen production for dermal testing. All four organisms will be used for sensitizing guinea pigs in order to obtain profiles of reactivity of mycobacterial antigens. Potency of antigens will be determined in homologously sensitized guinea pigs.

Major Findings: Work is too premature to report any findings at this time.

Significance to Biomedical Research and the Program of the Division: The in vitro lymphocyte transformation test correlated well with the skin test. The number of skin tests that can be performed is limited and suppression of reactions may occur. These disadvantages are overcome by the in vitro test which is perhaps more reliable in determining the sensitivity status of individuals. Since the reactivity of the various mycobacterial antigens is greatest in homologously sensitized animals, profiles of reactivity of an antigen in groups of animals sensitized with several mycobacterial species could provide a satisfactory identity test. The studies are directly related to the development and standardization of antigens with greater specificity in order to differentiate infections due to M. tuberculosis from those due to other mycobacteria.

Proposed Course of Project: To continue the projects as outlined above. It is hoped that analyses of the antigenic make up of some of the mycobacterial antigens might offer some clues as to which preparations have the greatest complement of unique antigens. Attempts will be made to isolate specific skin reactive reagents in pure form.

Honors and Awards: None

Publications: None

Serial No. DBS 160
1. Bacterial Products
2. Mycobacterial and Fungal Antigens
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Delayed Skin Reactivity and Lymphocyte Transformation in Monkeys with Experimental Allergic Encephalitis (EAE)

Previous Serial Number: NONE

Principal Investigators: Sotiros D. Chaparas, Ph. D.
Ronald Clark, Ph. D. (LP)
Other Investigator: Sally R. Hedrick

Cooperating Unit: Laboratory of Pathology

Man Years

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description:

Objective: To demonstrate in vivo and in vitro delayed hypersensitivity to central nervous tissue in EAE.

Methods Employed: Monkeys were sensitized with guinea pig brain tissue suspended in Freund's adjuvant containing BCG organisms. Skin tests and lymphocyte transformation were performed with tuberculin and a basal protein prepared by Dr. Marian Kies by extraction from guinea pig brains.

Major Findings: Monkeys were skin sensitive to both the basal protein and to tuberculin. Peripheral leucocytes were transformed by these antigens.

Significance to Bio-Medical Research and the Program of the Division: The results are in support of a delayed hypersensitivity mechanism for EAE. Lymphocyte transformation appears to be a useful adjunct in the study of this disease.

Proposed Course of Project: To test the efficiency of other mycobacterial species as adjuvants in the induction of EAE.

Honors and Awards: None

Publications: None

Serial No. DBS 16
1. Bacterial Products
2. Mycoplasma
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Role of Transitional L-Forms of Bacteria in Human Disease

Previous Serial Number: SAME

Principal Investigators: Michael F. Barile, Ph. D.
Thomas C. Francis, D.D.S. (NIDR)
Edward A. Graykowski, D.D.S., M.D. (NIDR)

Other Investigators: Joost J. Oppenheim, M.D. (NIDR)

Cooperating Unit: National Institute of Dental Research

Man Years

Total:	0.05
Professional:	0.05
Other:	0.0

Project Description:

Objectives: To identify and characterize the transitional L-forms of streptococci isolated from aphthous lesions and to examine their role in the pathogenesis of recurrent aphthae.

Methods Employed: Human and guinea pig lymphocyte cultures were inoculated with various antigens from pathogenic and non-pathogenic streptococci and incorporation of tritiated thymidine was determined.

Major Findings: (1) The mitotic response of unselected human lymphocyte cultures was greater to beta-hemolytic pathogenic streptococci than to alpha-hemolytic non-pathogenic streptococci. (2) Cell wall extracts of streptococci containing group specific carbohydrates and purified type M protein were better stimulants than were the intact streptococcal cells. (3) Pathogenic Group A streptococci did not stimulate cultures of lymphocytes from normal guinea pigs but did stimulate cultures of lymphocytes from immunized guinea pigs.

Significance to Bio-Medical Research and the Program of the Division: These studies will be useful in establishing the etiology of recurrent aphthae and, subsequently, may provide a means by which this disease may be arrested perhaps by vaccination. These studies are related to prevention and detection

of Mycoplasma in biologics and provide suitable materials to test new and improved methods for primary isolation and detection of Mycoplasma and L-forms of bacteria.

Proposed Course of Project: To pursue studies to improve methods for isolation and detection of L-forms of bacteria in biologics. To prepare findings for publication.

Honors and Awards: None

Publications:

Francis, T. C., Oppenheim, J. J., and Barile, M. F.: Lymphocyte Transformation of Streptococcal Antigens in Guinea Pig and Man. In Ricke, W. O. (Ed.): Proceedings of the Third Annual Lymphocyte Culture Conference, New York, New York, Appleton-Century-Crofts, Inc. 1969, pp. 501-518. (Reported as "In Press" in 1968).

Barile, M. F.: Variant Bacteria and Chronic Disease, Hospital Practice 3: 41-51, October 1968.

Serial No. DBS 17
1. Bacterial Products
2. Mycoplasma
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Mycoplasma in Human Disease and Biologics

Previous Serial Number: SAME

Principal Investigator: Michael F. Barile, Ph. D.

Other Investigators: Brigid G. Leventhal (NCI)
Richard A. DelGiudice (HRI)
Theodore R. Carski (HRI)
Clarence J. Gibbs (NINDB)
Anthony J. Morris (LVR)
Ruth L. Kirschstein (LP)
Stanley H. Singer (LP)
Herbert M. Yamashiroya (IITRI)
John E. Verna (MI)
Donald B. Riggs
Marion W. Grabowski

Cooperating Units: Medicine Branch, NCI
Huntingdon Research, Inc., Baltimore, Md.
Collaborative and Field Research, NINDB
Laboratory of Virology and Rickettsiology
Laboratory of Pathology
Illinois Institute of Technology Research Institute
Melpar, Inc.

Man Years

Total:	1.9
Professional:	0.35
Other:	1.55

Project Description:

Objectives: (1) To examine the biology, metabolism, and pathogenicity of Mycoplasma. (2) To develop new methods or improve existing methods for primary isolation and detection of Mycoplasma as they pertain to the test for Mycoplasma required for certain biologic products. (3) To investigate the role of Mycoplasma in diseases of man and animals.

Methods Employed: (1) Several different broth and agar media were employed and cultures were incubated aerobically and in a carbon-dioxide-nitrogen atmosphere. (2) Human lymphocyte cultures were inoculated with phytohemagglutinin (PHA) and PHA in combination with cultures of 6 Mycoplasma species, respectively, and the effects on the incorporation of tritiated thymidine were determined.

Major Findings: Subproject 1. A new species of Mycoplasma, designated as M. arginini, was isolated directly from sheep, mouse and goat tissues and from cell cultures derived from human, chimpanzee and dog tissues.

Subproject 2. The incidence and species designation of Mycoplasma contaminants isolated from primary and continuous cell cultures was established.

Subproject 3. PHA-induced transformation of human lymphocyte cultures was inhibited by arginine-using Mycoplasma but not by dextrose-using Mycoplasma. Inhibition was prevented with addition of arginine, or with complete medium but not by arginine-free medium. Arginine depletion by certain Mycoplasma deprives lymphocyte cultures of this essential amino acid resulting in impaired lymphocyte function.

Subproject 4. Hamster cell cultures infected with Mycoplasma hyorhinis and M. arginini were capable of yielding significantly greater amounts of vesicular stomatitis virus and Semliki Forest virus (SFV). These mycoplasma strains caused a pronounced decrease in the amount of interferon induced by a virus (SFV) and the amount of viral resistance induced by a synthetic complexed RNA (polyinosinic and polycytidilic acid) (with Drs. Singer and Kirschstein).

Subproject 5. Seventy (70) mycoplasma strains were isolated from 52 of 142 pharyngeal and urogenital tissues obtained from African Green monkeys (Cercopithecus aethiops). Sixty-six (66) of the 70 simian strains were identical or closely related to 5 established Mycoplasma species of man and include M. orale 2, M. orale 3, M. fermentans, M. salivarium, and strain Navel. Four simian strains were unrelated to the established Mycoplasma species of man and animals (Contract collaborative work).

Subproject 6. Twenty-one (21) strains representing 20 Mycoplasma species were inactivated by photodynamic action. The rate of inactivation was dependent on pH, concentration of the dye and the number of mycoplasma, the species of Mycoplasma and the suspending menstruum.

Significance to Bio-Medical Research and the Program of the Division: These studies may provide information on the pathogenicity of mycoplasmas for man. The improved culture procedures developed for the isolation of mycoplasma from human specimens are applicable to the isolation and detection of mycoplasmas in biologics which are subject to mycoplasma-contamination risk.

Proposed Course of Project: To pursue studies to improve methods for primary isolation of Mycoplasma from biologics and from tissues associated with disease. To consider assay methods for requirements for Mycoplasma vaccine products.

Honors and Awards: None

Publications:

Barile, M. F.: Mycoplasma and Cell Cultures. In Merchant, D. (Ed.): Cell Culture for Virus Vaccine Production, J. Nat. Cancer Inst. Monograph No. 29 pp. 201-204, 1968 (Reported as "In Press" in 1968).

Barile, M. F., Leventhal, B. G., Possible Mechanism for Mycoplasma Inhibition of Phytohemagglutinin-induced Lymphocyte Transformation. Nature 219:751-752, August 1968.

Barile, M. F., Delgiudice, R. A., Carski, T. R., Gibbs, C. J. and Morris, J. A., Isolation and Characterization of Mycoplasma arginini spec. nov. Proc. Soc. Exp. Biol. Med. 129:489-494, November 1968.

LBB

Annual Report of the
Laboratory of Biophysics & Biochemistry
Division of Biologics Standards
July 1, 1968 through June 30, 1969

Detection and characterization of viruses: Studies this year were designed to correlate the physical and biological properties of viruses, to develop more specific and sensitive methods for detecting, identifying and classifying viruses, and to study the in vitro interaction of recently isolated canine and simian cell adventitious agents with human cells. These studies involve, primarily, abortive virus-cell interactions, such as infectious canine hepatitis virus and simian cytomegaloviruses in human cells. The incidence, identity and characteristics of adventitious agents occurring in primary cell types used in live vaccine production were the main targets of study.

Serial No. DBS 86
1. Biophysics & Biochemistry
2.
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Detection and characterization of viruses

Previous Serial Number:

Principal Investigator: K. O. Smith, Ph. D.

Other Investigators: W. D. Gehle
M. D. Trousdale
J. F. Thiel
T. E. Hobbins, M.D.
R. C. Dunlap, Ph. D.
J. M. Nelson, M.D.
R. J. Hirschman, M.D.
L. F. Barker, M.D.
G. D. Clark
J. T. Newman
E. Harvey
A. Palmer, D.V.M.

Cooperating Units: Laboratory of Pathology
Laboratory of Viral Immunology
Melpar, Incorporated
National Institute of Arthritis & Metabolic Diseases
N.I.H. Animal Farm

Man Years:

Total: 9
Professional: 3
Others: 6

Project Description:

Objectives: (1) To correlate physical and biological properties of viruses; (2) To develop more specific and more sensitive methods for detecting, identifying and classifying viruses; (3) To study the in vitro interaction of recently isolated canine and simian cell adventitious agents with human cells. These studies involve primarily abortive virus-cell interactions, such as infectious canine hepatitis virus and simian cytomegaloviruses in human cells.

Methods Employed: Cell culture techniques, high resolution electron microscopy, virus particle counting, complement fixation reactions, precipitin reactions, agglutination reactions, fluorescence microscopy, and virus infectivity titrations in tissue cultures.

Major Findings: (1) Development and application of a method for detecting small amounts of virus or virus-infected cells (with Warren D. Gehle).

As an aid in recognizing the presence of small quantities of viruses and identifying them by electron microscopy, we have designed a routine, simple method for pelleting virus-infected cells or cell-free virus in the tips of small, pointed, commercially available plastic capsules. Virus material which is deposited at the tip of the capsule, remains in a fixed position throughout fixation, dehydration, plastic embedding and sectioning. This procedure eliminates many of the mechanical problems previously associated with pelleting and plastic embedding, and allows excellent results when quantities of viral material are small and when virus concentrations are low. The procedure was successfully used as an aid in identifying viruses occurring naturally in fetal calf serum, duck cell cultures and in primary African green monkey kidney cell cultures.

(2) Cytomegaloviruses as common adventitious contaminants in primary African green monkey kidney cell cultures (with John F. Thiel, Joseph T. Newman, Estelle Harvey, Melvin D. Trousdale, Warren D. Gehle, and Glennelle D. Clark).

Twenty-seven of fifty-two lots of primary African green monkey (AGM) kidney cell cultures were found to contain cytomegalovirus (CMV). The cell cultures, all derived from apparently healthy animals, required long incubation periods before CMV could be detected. Less than half the contaminated cultures showed cytopathic effects before 4 weeks. About one-fifth of the contaminated cultures failed to show CPE during a 40-day observation period; CMV was initially recognized in these cultures by fluorescence microscopy. Fluorescence and electron microscopy of CMV-infected cells revealed highly distinctive nuclear inclusions. All virus strains tested showed typical focal CPE on monolayers of WI-38 cells after 5 days. Nearly all AGM sera tested contained complement-fixing antibody against several simian CMV strains. Substantial levels of neutralizing antibody against one CMV strain were present in AGM sera tested. Isolation and serological data indicate that CMV is widely distributed as a latent infection among apparently healthy African green monkeys. This wide distribution in well-conditioned monkeys might be considered when the significance of herpes-like viruses in primate cells is interpreted. Simian CMV-human cell interactions are of interest because of the well-known association of herpesviruses with oncogenic processes and because simian CMV strains replicate slowly in human cells.

(3) Some properties of cytomegaloviruses isolated from primary African green monkey kidney cell cultures (with Melvin D. Trousdale, principal investigator)

Recent studies have shown that cytomegalovirus (CMV) is a common adventitious contaminant in primary African green monkey kidney (AGMK) cell

cultures. The frequency with which this virus appears as a contaminant in primary cell culture warrants concern due to the widespread use of AGMK cell cultures in vaccine production and basic virological research. Because of the limited knowledge of simian CMV and the availability of a large number of uncharacterized CMV strains, a study concerning different properties of these viruses was undertaken. We studied comparative assays of the agents in cell cultures, growth characteristics, inclusion body and plaque formation, host cell range, thermal stability, and their serological relationship to other herpes agents.

Studies were carried out on isolates of African green monkey (AGM) cytomegalovirus (CMV), common contaminants in primary AGM kidney cell cultures. Host spectrum experiments included the following cells: human, AGM, and rhesus fibroblast; primary human embryonic kidney (HEK), AGM kidney, and rabbit kidney (RK); human amnion (HA); duck embryo (DE); and BHK-21. After several days incubation, infectious virus was detected in the human, AGM, and rhesus fibroblast and the primary HEK, AGM, and RK. Best virus growth occurred in human lung fibroblasts. Human amnion, DE, and BHK-21 appeared to be "dead-end" hosts.

Plaque assay and tube titrations were utilized to determine infectivity titers. Results of the plaque method could be read after about 5 days, while 30 days were required for the tube titration. In parallel experiment, the number of infectious units/ml determined by the two methods were almost identical. The effect of sonic oscillation on simian CMV aggregates was examined. Titers were increased by brief sonic treatment, but prolonged sonication reduced the titers. Stability of infectious virus during storage proved to be temperature dependent and greatly enhanced by the presence of 7% dimethyl sulfoxide (DMSO). The infectivity of CMV stocks stored at -270 C and -70 C was greater than those stored at -20 C and 0°C.

The rate of replication of AGM CMV in human lung fibroblast cells was similar to that previously found in studies with human CMV. AGM CMV were relatively slow growing agents. The production of CF antigens preceded the synthesis of infectious virus and the appearance of CPE. Virus neutralization tests indicated a serological relationship between CMV isolates and simian agent-6. No relationship could be demonstrated with herpes simplex virus, human CMV, simian agent-8, or monkey B-virus.

(4) Abortive infection of human cell cultures by a canine adenovirus (with Warren D. Gehle, principal investigator).

The successful preparation of several vaccines using tissue culture propagated virus indicates that in the future many viral vaccines may consist of virus grown in cell cultures initiated from human or animal tissue. The major objection to the use of such cell culture systems is the presence of adventitious agents which may be potentially dangerous to humans. Since a live measles vaccine is currently being prepared in primary canine kidney cells, and since infectious canine hepatitis virus (ICHV) has been isolated

as an adventitious agent from primary canine kidney cells, it was of interest to study the in vitro synthesis of ICHV in human cells.

Serial passage of ICHV in human cells resulted in a rapid decrease in infectious virus, but a relatively slow decrease in CF antigen production. If the amount of input infectious virus is taken as a reference, calculations indicate that the ratio of CF antigen per input infectious unit increased with each serial passage. This phenomenon was most noticeable after the first serial passage in human cells, or when low multiplicities of infection with ICHV were used. Viral antigens and infectious virus recovered from human cell cultures were found to be the result of de novo synthesis. Although the mechanism of the nonparallel production of viral antigens and infectious virus in human cells is unknown, other evidence indicates that many of the ICHV particles may be defective for human cells; that is, they are capable of inducing only the synthesis of CF antigen and not the synthesis of infectious virus.

While ICHV has not as yet been associated with human disease, the findings that ICHV interacts with human cells to produce substantial amounts of tumor antigen, viral antigen, and low levels of infectious virus make it clear that ICHV should be considered potentially dangerous to humans. Consequently, cell cultures initiated from canine tissues should be tested for the presence of ICHV prior to being used in vaccine production.

(5) Search for infectious agents in canine cell cultures by fluorescence microscopy; their identification by standard virological techniques and by electron microscopy (with Ruth C. Dunlap, Ph. D., J. F. Thiel, Glennelle D. Clark, John T. Newman and Amos Palmer, D.V.M.).

Canine kidney cells were selected for further study because of recent reports that viral agents could be readily isolated from dog organs, because not very much is known about canine adventitious agents, because live measles vaccine is now made in canine kidney cells, and because live rubella vaccine may be produced in these cells. It was hoped that (a) useful information could be obtained concerning the incidence of canine agents in kidney cells, (b) the fluorescence antibody screening technique could be evaluated in another large scale study, and (c) minimum requirements for adventitious canine virus detection could be determined.

A total of thirty dogs were obtained from Dr. Amos Palmer who is located at the NIH Animal Farm. These dogs were selected from a group of several hundred animals on the basis of their general poor health (many were acutely ill). Selection of diseased animals was made in order to increase the likelihood of isolating viruses from their organs. Primary kidney cell cultures were obtained from all thirty dogs, liver cultures were obtained from twenty-nine and pancreas cultures were obtained from two animals. Cytopathic effects (CPE) developed in many cultures; the remainder were held for a minimum of 35 days before examination by fluorescence microscopy and electron microscopy. The study is incomplete, but fluorescence microscopy gave positive results in about four-fifths of the cultures; electron microscopy revealed viruses in

about two-thirds of the cultures examined. Preliminary results indicate the presence of adenoviruses, herpesviruses, myxoviruses, and two unclassified agents. The agents isolated are being studied in terms of the minimum testing requirements needed for detection if they should be present in primary canine kidney cells used for vaccine production.

(6) Relationship between Australia antigen and human hepatitis (with Lewellys F. Barker, M.D., as principal investigator, Warren D. Gehle, Richard J. Hirschman, M.D., and N. Rhael Shulman, M.D.).

LBB has supported Dr. Lewellys F. Barker in his effort to establish an active research program on human hepatitis. The recent reports on the relationship between Australia antigen and human hepatitis led us to study Australia antigen as (1) a possible etiologic agent of hepatitis and/or (2) a possible indicator of infectious material. Our primary goal was to establish a competency in this field so that eventually blood products could be tested for the presence of hepatitis virus.

The appearance of Australia antigen during the incubation period of post-transfusion hepatitis, reported by others, suggests that the antigen is hepatitis virus itself and virus-like particles have been found in antigenic sera. We have found the "Australia" antigen in 74% of 62 prospectively studied patients who developed clinical hepatitis following exposure to contaminated blood products and have documented the temporal relationship between clinical disease and antigenemia. In a common source outbreak of infectious hepatitis and in patients with infectious hepatitis in an endemic area, occasional sampling showed incidences of antigenemia of only 5% and 16%. Two of 128 healthy chimpanzees and one of 14 healthy gibbons also had the antigen.

The "Australia" antigen appears to be a virus-like particle on examination by electron microscopy and the antigens found in serum hepatitis, infectious hepatitis and in nonhuman primate blood were indistinguishable by both morphologic and immunologic criteria.

Antibody to "Australia" antigen was found in 8 of 29 hemophiliacs who had been treated in a routine manner with multiple transfusions, and in 2 chimpanzees which had been transfused repeatedly with chimpanzee blood.

The 20 mμ particles associated with Australia antigen in the plasma of hepatitis patients have a density in CsCl of 1.27 to 1.28 after lipid extraction. Plasma which had been stored for 17 years at -20°C contained particles with the same density after lipid extraction. EM examination revealed spherical virus-like particles with a 2 mμ outer coat which is removed by ether treatment.

(7) Search for adventitious agents in primary human kidney and heart cell cultures (with John Nelson, M.D., principal investigator).

Recent studies, utilizing improved virological detection techniques, have demonstrated the presence of latent viral agents in a variety of mammalian and avian tissues. In order to determine whether a similar

situation exists in man, primary cultures of human kidney and heart tissue have been screened for adventitious agents. Preliminary results are as follows:

A-Primary Human Kidney (PHK) - Over the past five months, twenty autopsy specimens have been obtained from the Bethesda Naval and the Walter Reed Army Hospitals. The patient population consisted of fourteen males and six females, ranging in age from thirteen years to seventy-one years (average age - forty-one). Causes of death varied from acute infections or traumatic episodes to terminal malignancies. The only individuals excluded from the study were those with obvious renal pathology.

Transverse sections from either kidney were washed, trypsinized, and seeded into 250 cc. Falcon flasks. Adjacent sections were frozen intact at -70°C. for future reference. The time interval between the patient's death and the initiation of the trypsinization prodedure ranged from five to forty-eight hours. Successful tissue cultures were directly related to the cause of death (acute trauma or myocardial infarction) and the time elapsed since death (less than sixteen hours). Eleven of the cultures were classified as early failures, with either no growth (five specimens) or gross contamination (six specimens) manifest within the first week. Two additional specimens were listed as late failures, with either gross contamination or limited cell growth noted after two weeks.

Six cultures have been maintained for a minimum of thirty-two days. Five of these manifested minimal to moderately pronounced CPE after twenty-four to thirty-three days. Four different specimens have been tested with the indirect Coons' reaction, utilizing fluorescein conjugated anti-human gamma globulin and fluorescence microscopy. Sera samples consisted of a battery of six human sera pools (collected from two or three healthy adult patients), pooled human gamma globulin, and the patient's own sera, if available. All four specimens demonstrated positive fluorescence with their own sera and each of the pooled sera; none showed any reaction with the pooled gamma globulin. Positive controls consisted of Adeno-2 infected HA cells, which reacted with all of the sera samples. Negative controls, consisting of HEK cells, were contaminated prior to use and must be repeated. Representative samples of positive cultures have been photographed; fluorescence in the PHK cells appears to be localized in the perinuclear and cytoplasmic regions. Acridine orange preparations have not revealed any inclusion bodies. Further attempts at characterization, utilizing electron microscopy (thin sections and negative staining) and passage of primary material into HA cells, are now in progress.

B-Primary Human Heart (PHH)- Samples of either ventricular or auricular tissue were obtained from twelve of the above patients. The study p· pulation consisted of ten males and two females, ranging in age from seventeen years to fifty-six years (average age - forty-one years). The time interval between the death of the patient and the initiation of the tissue cultures ranged from five hours to seventeen hours. Seven samples were trypsinized in

identical fashion to the kidney specimens; the remaining five were "explanted" into 30 ml. Falcon flasks. Only one specimen has been carried beyond thirty days. Three of the trypsinized specimens and all five explants succumbed to gross contamination within five days. Three trypsinized specimens showed no growth after one week; two others were discarded after several weeks because of limited growth.

(8) Search for indigenous viral agents in primary cell cultures of ducks (with John F. Thiel, Glennelle D. Clark, Warren D. Gehle, and Joseph Newman).

In order to develop our methods for detecting latent indigenous duck viruses, we have gone first to the materials most likely to contain such viruses, i.e., adult birds from farms where no isolation precautions are known to be used. Thus we hoped to isolate and study common latent duck agents and to acquire competency for later examination of duck embryo cell cultures, (which one might expect to be less frequently contaminated with latent indigenous viruses). Some of the adult Pekin ducks originated from Truslow Farms.

The results of these studies are incomplete, however several duck liver and kidney cultures, on long incubation, have shown spontaneous degeneration characteristic of virus-infected cultures. The most common type cytopathic effect is a foamy, vacuolating type which involves the cytoplasm. Fluorescence microscopy of these cultures with the ducks' own sera is positive. Electron microscopy revealed virus-like particles in the cytoplasm having diameters between 25 and 50 mμ. Studies are in progress to determine whether these are virus infected cultures.

Embryonic duck cells from Truslow Farms, when held for over 30 days, developed occasional foci of foamy CPE, which could be easily recognized by fluorescent antibody microscopy or acridine orange staining. Some cultures showed fluorescence in areas without apparent CPE; occasional cells showed nuclear fluorescence as well as cytoplasmic fluorescence. The significance of these findings is under study, but it is tentatively concluded that some of these observations are due to latent virus infection of duck cell cultures. Studies in progress include attempts to passage the positive materials to virus-free cell cultures and to identify the possible agents serologically.

Significance to Bio-Medical Research and the Program of the Division: Information concerning the incidence, identity and characteristics of adventitious agents occurring in primary cell types used in live vaccine production is important to the Division.

Proposed Course of the Project: Some of the projects will be discontinued because of the departure of Dr. Smith.

Honors and Awards: None

Publications:

Hobbins, T. E. and Smith, K. O.: Fluorescent plaque assay of rubella virus. Proc. Soc Exp. Biol. & Med. 129: 407-412, 1968.

Smith, K. O. and Hobbins, T. E.: Physical characteristics of rubella virus. J. Immunol. (In press)

Gehle, W. D. and Smith, K. O.: Abortive infection of human cell cultures by a canine adenovirus. In press.

Hirschman, R. J., Shulman, N. R., Barker, L. F. and Smith, K. O.: Virus-like particles in sera of patients with hepatitis and in simian sera. J.A.M.A. In press.

Smith, K. O.: Proceedings, First Internat. Congress of Virologists, Helsinki, 1968.

Smith, K. O., Thiel, J. F., Newman, J. T. Harvey, E., Trousdale, M.D., Gehle, W. D. and Clark, G.: Cytomegaloviruses as common adventitious contaminants in primary African green monkey kidney cell cultures. J. Nat. Cancer Inst. 489-497, 1969.

Smith, K. O. and Gehle, W. D.: Pelleting viruses and virus-infected cells for thin-section electron microscopy. Proc. Soc. Exp. Biol. & Med. In press.

LBBP

Annual Report of the
Laboratory of Blood and Blood Products
Division of Biologics Standards
July 1, 1968 through June 30, 1969

During this fiscal year two major objectives were realized by the staff of the Laboratory of Blood and Blood Products. Current technical standards have been developed for all licensed blood products and as rapidly as these can be processed, they will be published as proposed Additional Standards. Secondly, an analysis of all control tests performed in the Laboratory of Blood and Blood Products during the past calendar year was completed. It is planned to utilize a computer so that a profile of the tests will be readily available and they can also serve as a guide for designing future control activities.

In addition to continued work with blood components and fractions widely in use in modern medical therapy, the Laboratory is attempting to anticipate new demands that are imminent because of recent medical developments. The increased use of organ transplants has emphasized the need for immunosuppressants and tissue typing reagents for the successful accomplishment of these surgical procedures. The Laboratory has been involved in developing a project team, under the leadership of Dr. Vito M. Esposito, to begin active investigation of anti-lymphocyte preparations and tissue typing reagents in expectation of licensure requirements.

The continued shortage of personnel necessarily has affected the depth and scope of the activities of the Laboratory. Because certain regulatory functions can not be curtailed, the impact has been reflected in a reduction of back-up research and long-range projects.

I. Intramural Research Activities: The research program of the Laboratory embraced several major areas - the preparation and characterization of blood products, including those of the coagulation and fibrinolytic systems, investigations of their stability and potency, and standardization and improvement of assay techniques. Products to which attention was directed included immunoglobulins and related preparations, grouping and typing sera, albumin, plasminogen, and Factors I, II, VIII, IX, X and XIII of the coagulation system.

II. Other Than Research Activities: One important and demanding assignment of this Laboratory is the review of establishment and product applications relating to blood. Approximately 75% of all licensing requests received by the Division are referred to the Laboratory of Blood and Blood Products to review and recommend action. This year there was a record input. Eleven establishment applications, 68 product applications, and 95 amendments were received. See Table I. A committee of staff members is selected to review each request. The time required to complete this review and to make appropriate recommendations for subsequent action necessarily is dependent upon the type of application or the degree of modification represented by the amendment.

Another regulatory function routinely performed in this Laboratory is control testing of blood and blood products prepared by licensed establishments. These products are provided to the Division either prior to the release of manufactured lots, or as randomly selected inspection samples. 3751 samples were subjected to 20,358 tests to determine that standards of purity, potency, and safety had been met. See Table II.

In addition, each Section has prepared detailed information on its control testing during the past year, with summarized results and suggestions for future control programming.

Work on updating existing technical standards and preparing standards for products previously licensed with only minimum requirements has been one of the major undertakings of the past year. On March 13, 1969, the Notice of Proposed Rule Making for Additional Standards for Plasma (Human) and for Red Blood Cells (Human) was published. These standards took cognizance of the earlier action by the Director to prohibit the pooling of Plasma (Human), excepting only Antihemophilic Plasma, and for the first time allowed plasmapheresis to be used as a source of Plasma (Human).

Other proposed technical standards that were completed and submitted for informal comment prior to publishing in the Federal Register included radioisotope labeled biologicals, Rh_0 (D) Immune Globulin (Human), Cryoprecipitated Antihemophilic Factor (Human), and Whole Blood (Human), Antihemophilic Factor Removed. As quickly as other standards are processed they will also be distributed.

10 of the 75 pending license applications are for biologics not previously licensed. Development of technical standards for these products must be coordinated with the processing of the applications so they will be available at the time the license is granted. Manufacturers and other interested investigators have been most helpful in providing data and in constructively offering suggestions as these standards are developed. Two new products licensed during this year were Vaccinia Immune Globulin (Human) and Factor IX Complex (Human).

Eight inspectors and five inspector trainees, appointed from this Laboratory, participate in the annual inspection of licensed establishments. A total of 247 man-days were spent on this activity, and 339 locations were visited. See Table III.

A continuous in-staff training program is conducted for this group of inspectors. In addition to monthly meetings, a special orientation course was designed and given to the newly designated trainees.

At the request of the Director, Dr. John Finlayson served as consultant to the Laboratory of Biophysics and Biochemistry from June 1968 to January 1969.

Members of the donor panel continue to provide an important service to the control testing of diagnostic reagents. Approximately 700 blood samples were drawn from specially selected donors by the Laboratory staff. The cooperation of these willing donors and of the Clinical Center Blood Bank whose facilities are used for this procedure, is very much appreciated.

Status of License Applications
Laboratory of Blood and Blood Products
Division of Biologics Standards
April 1, 1968 through March 31, 1969

TABLE I

	Establishment Applications	Product Applications	Amendments	Total
No. Pending - 4-68	13	65	33	111
No. Received	11	68	95	174
No. Recommended	11	65	58	140
No. Withdrawn or Inactive		6	9	15
No. Pending - 3-69	13	62	61	136

Summaries of Certain Control Activities
Laboratory of Blood and Blood Products
Division of Biologics Standards
April 1, 1968 through March 31, 1969

TABLE II

CONTROL TESTING OF BLOOD AND BLOOD PRODUCTS

Type of Products	Number of Lots Tested				Number of Tests			
	Inspection Sample	For License	For Release	TOTAL	Purity	Potency	Safety	TOTAL
Blood Derivatives	9	37	1769	1815	951	484	1293	2728
Diagnostic Serums	101	95	1142	1338	7646	4406		12052
Reagent Red Blood Cells	13	12		25	119	6	30	155
Whole Blood, Plasma, Packed Red Blood Cells	524	49		573			5423	5423
	647	193	2911	3751	8716	4896	6746	20358

Summaries of Certain Control Activities
Laboratory of Blood and Blood Products
Division of Biologics Standards
April 1, 1968 through March 31, 1969

TABLE III

CONTROL ACTIVITIES REQUIRING TRAVEL STATUS

Type of Activity	Locations Inspected	Contracts Involved	Man Days of Travel
Annual Inspections	339		247
Supportive of Contracts		5	9

Serial No. (NIH-DBS) 33
1. Blood & Blood Products
2. Blood & Blood Derivatives
Coagulation
Diagnostic Reagents
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Preparation, Characterization, Stability and Potency of Blood Products

Previous Serial Number: Same

Principal Investigators: Pinya Cohen, Ph.D.
John S. Finlayson, Ph.D.

Other Investigators: Ellis Dunn, B.S.
Elizabeth B. Paul, A.B.
Joan Roelands, B.A.
Peter C. Stang, A.B.
Clarence F. Szwed, M.S.
Donna Williams, B.S.
Andrew M. Young, M.S.

Man Years:
Total: 3.7
Professional: 3.0
Other: 0.7

Patient Days: None

Project Description:

Section I. Stability of Blood Products

Objectives: To determine the changes in properties of blood proteins which occur during processing, storage and related treatment.

Methods Employed: a) Studies on the effects of mild heating of Immune Serum Globulin (Human) were continued. (Finlayson, Young, & Hudson)

b) The study of the stability of Blood Grouping Sera at different storage temperatures was continued, as was the study of the stability of certain Anti-Rh Typing Sera, which was begun last year. (Szwed)

Major Findings: a) Ultracentrifugal studies carried out after storage for up to 2 years confirmed preliminary findings that proteolytic degradation of Immune Serum Globulin (Human) is greatly reduced, but not eliminated, by heating at 45°C or 50°C prior to storage. Short term

investigations revealed that whereas heating at 50°C for 72 hours produced changes readily detectable by electrophoresis or complement fixation, changes produced by heating at 45°C were minimal. Heating at either temperature resulted in a marked decrease in the amount of immunoglobulin dimer present.

b) Blood Grouping Sera stored for 4.5 years at 5°C exhibited no change in titer. This phase of the study was therefore terminated since 4.5 years exceeds the longest possible dating period. However, since evidence concerning the stability of such sera in the frozen state is conflicting, the portion of the study dealing with frozen sera will be continued.

Selected Anti-Rh Typing Sera for Slide Tests remained stable during storage for one year, regardless of whether the storage was carried out in plastic or glass containers. On the other hand, such sera intended for Saline Tube Tests showed a significant decline during the storage period. Additional studies are anticipated prior to recommending revised dating periods for these products.

Section II. Development of Methods and Standards

Objectives: To develop standardization of assay methods, to modify and extend existing assay techniques.

Methods Employed: a) A two-channel Auto-Analyzer(R) was used to measure potencies of Blood Grouping Sera and Anti-Rh Typing Sera proposed for automated grouping and typing. Levels of the additives employed in the tests were varied in an attempt to determine optimal reaction conditions. (Dunn)

b) Investigations of the potency test for Rh_o (D) Immune Globulin (Human) was continued utilizing the basic radioimmunoassay and the ancillary techniques described in the previous Annual Report. (Paul, Stang, Dunn & Williams)

c) Immune Serum Globulin (Human) of placental origin was screened for contamination with blood group specifc substances A and B. The degree of contamination was measured serologically by hemagglutination inhibition. (Hudson & Cohen)

d) Albumin and globulin of placental and venous origin were compared immunologically. The number and types of components of these preparations were determined with adsorption, gel diffusion and immunoelectrophoretic techniques. (Roelands & Cohen)

Major Findings: a) Tests carried out on NIH reference antisera and commercially prepared grouping and typing sera showed that reliable test results, comparable with those reported by manufacturers, could be obtained with the Auto-Analyzer(R). Optimal levels of bovine albumin and

polyvinylpyrrolidone were determined for the test systems studied; the level of bromelin could be varied over a wide range without affecting the results. These findings demonstrated the capability of the Laboratory to evaluate the potency of such sera, but indicated the need for a multi-channel analyzer if routine testing is to be technically feasible.

b) Certain of the factors affecting results in the potency test for Rh_o (D) Immune Globulin (Human) were established. These included the particular lot of bovine albumin used as a diluent and the extent of denaturation incurred during radiolabeling of the protein and removal of the excess radioisotope. This knowledge was shared with manufacturers of this product in a series of demonstration sessions. In addition a simpified, single-dilution test was developed. It has the following advantages: 1) only 1 ml of test material was needed; 2) simple hand calculations replaced the computerized data processing; and 3) four to six lots of Rh_o (D) Immune Globulin could be analyzed simultaneously for antibody concentration.

c) Immune Serum Globulin (Human) of placental origin from various manufacturers contained from 10 to 200 µg/ml of B substance and from 1 to 20 µg/ml of A substance. Lots of mixed (venous plus placental) origin contained B substance at levels ranging from 1 to 50 µg/ml, whereas lots of venous origin were free of blood group substances. Initial studies with Tetanus Immune Globulin (Human) showed low levels of contamination with B substance despite the fact that this material is of venous origin (hyperimmune donors). The blood group substance may come from the Immune Serum Globulin (Human) used to dilute the Tetanus Immune Globulin (Human).

d) Preliminary experiments have revealed that 1) there are components in Immune Serum Globulin (Human) of placental origin which are not found in that of venous origin and 2) Normal Serum Albumin (Human) of placental origin is less antigenic (in rabbits) than that of venous origin. Furthermore, gel diffusion tests showed that some normal rabbit sera reacted nonspecifically with a component in Normal Serum Albumin (Human).

Significance to Bio-Medical Research and the Program of the Division: The Laboratory of Blood and Blood Products is responsible for control of the safety, purity and potency of products derived from blood. Such surveillance involves the possession of knowledge regarding the stability of biological products as well as the availability of appropriate control test methods and technical standards. This project fulfills these needs by providing information leading to more realistic dating periods and control test methods.

Proposed Course of Projects: This is a continuing project.

Honors and Awards: None

Publications: None

Serial No. (NIH-DBS) 81
1. Blood & Blood Products
2. Blood & Blood Derivatives
Coagulation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Clotting and Fibrinolytic Systems

Previous Serial Number: Same

Principal Investigator: David L. Aronson, M.D.

Other Investigators: B. Lynne Ehrenreich
John S. Finlayson, Ph.D.
Herman Hudson
Anne J. Mustafa
Mervin Taylor, B.S.
Andrew M. Young, M.S.

Cooperating Units: National Heart Institute (Dr. John J. Pisano)
National Institute of Arthritis and Metabolic Diseases (Dr. William R. Carroll)

Man Years:
Total: 3.7
Professional: 2.1
Other: 1.6

Patient Days: None

Project Description:

Objectives: To purify and characterize components of these systems and to develop an understanding of their chemistry as a basis for more useful assay procedures.

Methods Employed: a) Physical studies of purified human Factor X, prepared by methods previously developed in this Laboratory, were carried out in assocation with Dr. William R. Carroll. The major instrument used in these studies was the analytical ultracentrifuge. (Young & Aronson)

b) The sequence of events in the activation of human Factor X was investigated with the aid of gel filtration on Sephadex(R) G-100 and G-200 and ion exchange chromatography on DEAE cellulose. Further studies dealing with the chromatographic separation of human Factors II and X have been carried out. (Aronson & Mustafa)

c) A cooperative study has been undertaken on the utilization of the present Antihemophilic Factor (Human) (Factor VIII) reference preparation as a tentative standard for Factor IX. Attempts have been made to isolate a Factor IX complex preparation which could serve as a prototype for evaluating the feasibility of employing such a preparation for standardizing the more purified Factor IX products. Development of a Factor IX assay which would be used for these products has been undertaken. Influences of Contact Factor, Factor VIII and other substances in the test system have been investigated. (Aronson & Mustafa)

d) A collaborative study, in which the World Health Organization participated, was carried out to compare the values obtained by tests for Factor VIII as performed in various laboratories. Samples of the new reference preparation were distributed and the reported results were compiled and compared with those obtained in this Laboratory. (Taylor & Aronson)

e) In order to have a method available for structural comparsion of proteins involved in coagulation, development and modification of the peptide mapping ("fingerprinting") technique was undertaken. (Young)

f) The viscometric assay of streptodornase was carried out under various conditions so as to achieve a precise comparison between the present U.S. unit and the International Streptodornase Unit. (Hudson & Finlayson)

g) Work with Dr. John J. Pisano, directed at confirming the nature of the crosslink in human fibrin polymerized by human Factor XIII, was continued. A chemical test for this type of crosslinking was developed and the chemical and enzymic methods were carried out in parallel on a series of samples including: fibrin contaminated with Factor XIII, fibrin free of Factor XIII, fibrin free of Factor XIII plus added Factor XIII. (Finlayson)

h) In order that the purity of plasminogen purified in this Laboratory could be compared with that isolated elsewhere, a detailed examination was made of certain aspects of the casein assay, including the substrate and the units employed. (Finlayson & Ehrenreich)

Major Findings: a) Human Factor X was found to have a sedimentation coefficient of 3.7 S and a diffusion coefficient of 5.5×10^{-7} cm^2/sec. These values are consistent with published data for bovine Factor X.

b) Activation of human Factor X in the presence of human tissue was found to yield two active fragments and three or four inactive fragments. A similar pathway appeared to be followed when the activation was carried out in the presence of citrate. Gel filtration on Sephadex(R) G-200 was capable of separating human Factors II and X, Factor X being eluted somewhat earlier. This result was obtained with purified preparations as well as with whole plasma, thus indicating a difference in molecular volume between these two proteins in addition to those differences demonstrated by other methods.

c) The potential Factor IX refernce preparation has been distributed in cooperation with the International Committee on Haemostasis and Thrombosis, to a number of laboratories. Some of these have already reported the Factor IX level of this preparation in terms of the level in a population of normal donors. A tentative assay for Factor IX has been achieved. It was found to yield results which were internally consistent but not necessarily in agreement with those obtained by other laboratories employing different methods. Chromatography on hydroxyapatite, which has proved successful in separating human Factors X and II, resulted in no separation of human Factors X, IX and, apparently, VII.

d) Factor VIII levels reported by most groups which tested the new refernce preparation agreed with those determined in this Laboratory. The preparation therefore may be considered to be satisfactory for use in inter-laboratory standardizations.

e) The peptide mapping technique has been modified so that it can be carried out with thin-layer (as opposed to paper) chromatography and electrophoresis, thus resulting in a considerable reduction in the time necessary for the chromatographic step. Investigations with well known proteins have indicated that the method in this form can be used to study protein structure as well as to detect structural alterations occurring during the course of enzymic activation.

f) By utilizing the level of Mg^{++} in the reaction mixture suggested by Lederle Laboratories and by raising the substrate concentration from the suggested value of 0.15% DNA to 0.6% DNA it was possible to obtain a linear increase in the reciprocal relative viscosity throughout the assay period. Under these conditions, assays of lots of commercial streptodornase as well as of two lots used as working standards yielded values in good agreement with those obtained by the manufacturer. The stated equivalence of 1.59 U.S. Streptodornase Units to 1.0 International Streptodornase Unit was confirmed. It therefore appears reasonable 1) to adopt the International Streptodornase Unit as the U.S. Streptodornase Unit and 2) to adopt the present U.S. Streptokinase Working Standard as the U.S. Streptokinase Working Standard.

g) The chemical tests developed for crosslinking involving ε-lysyl bonds consisted of cyanoethylation followed by acid hydrolysis and analysis for lysine. Under these conditions ε-amino-crosslinked lysine yields free lysine whereas lysine elsewhere in the protein molecule yields an N-carboxyethyl derivative. The levels of ε-amino-crosslinked lysine measured in this manner agreed with those of ε-(γ-glutamyl)lysine isolated after enzymic hydrolysis; they were greatly depressed in fibrin free of Factor XIII and were restored when Factor XIII was added. This agreement indicated 1) that lysine is not crosslinked to any acceptor other than glutamate and 2) that the cyanoethylation technique is a valid procedure for detecting ε-lysyl crosslinks in proteins.

h) The procedure which was developed in this Laboratory (Annual Report 1964 to 1965) for removal of β-casein by precipitation in the presence of urea was found to vary in effectiveness with the commercial casein used as starting material. The treatment generally increased the sensitivity of the casein as a plasmin substrate, but often failed to eliminate the lag period. Nevertheless, by suitable modification of the assay a constant ratio was obtained between the activity of streptokinase-activated plasminogen (prepared in this Laboratory) and that of spontaneously-activated plasmin (prepared by the Michigan State Department of Health), regardless of the casein - treated or untreated - used as substrate. The ratio of the units used for research assay purposes in this Laboratory and the so-called Michigan State caseinolytic unit varied somewhat with the substrate, but was constant when different batches of a given substrate casein were employed. The modified assay thus appears reliable, is not dependent on a single source of substrate, and yields values which can be compared directly with those obtained in other laboratories.

Significance to Bio-Medical Research and the Program of the Division: Control of the purity and potency of therapeutic blood derivatives used to treat coagulation and thrombotic disorders involves detailed understanding of the mechanisms of the clotting and fibrinolytic processes and the chemistry of the in vitro assay methods. This understanding is leading to improved validity and precision in control tests.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: Aronson, D. L. and Ménaché, D.: Action of human thrombokinase on human prothrombin and p-tosyl-L-arginine methyl ester. Biochim. Bioplys. Acta 167: 378-387, Oct. 1968.

Finlayson, J. S.: Heterogeneity of human fibrinogen revealed by hydroxyapatite chromatography. Abs. XII Congress, Int. Soc. Hematol. 173, Sept. 1968.

Lanchantin, G. F., Friedmann, J. A., Hart, D. W., and Aronson, D. L.: Studies on the mechanism of human prothrombin activation by Factor X. Fed. Proc. 28:639, Mar.-Apr 1969.

Pisano, J. J., Finlayson, J. S., and Peyton, M. P.: Cross-link in fibrin polymerized by Factor XIII: ε-(γ-glutamyl)lysine. Science 160: 892-893, May 1968.

Pisano, J. J., Finlayson, J. S., and Peyton, M. P.: Chemical and enzymic detection of protein cross-link. Measurement of ε-(γ-glutamyl)lysine in fibrin polymerized by Factor XIII. Biochemistry 8:871-876, March 1969.

Serial No. (NIH-DBS) 82
1. Blood & Blood Products
2. Coagulation
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Proteins in Body Fluids

Previous Serial Number: Same

Principal Investigator: John S. Finlayson, Ph.D.

Other Investigators: B. Lynne Ehrenreich
David M. Hudson, B.S.*

Cooperating Units: National Cancer Institute (Dr. Michael Potter)

Man Years:
Total: 0.7
Professional: 0.3
Other: 0.4

Patient Days: None

Project Description:

Objectives: To complement available data on blood proteins with a reservoir of basic information on the properties of and methods suitable for use on proteins of blood and other fluids.

Methods Employed: In association with Dr. Michael Potter the study of proteins of the body fluids of laboratory animals was continued. Since previous work in this Laboratory had shown that two codominant alleles controlling the electrophoretic variation (which is apparently due to a single alteration in the primary structure) of one of the components of the major urinary protein (MUP) complex of the mouse were on linkage group VIII, a study was undertaken to determine the precise location of this locus (the Mup-a locus). Mice from two inbred strains differing in MUP type, coat color and presence of the Pintail trait (all of which are controlled by loci on linkage group VIII) were bred. Members of the F_1 generation exhibiting the Pintail phenotype were backcrossed with members of one of the parental strains. The resulting offspring were examined for coat color and presence of the Pintail trait, whereupon urine was collected from individual mice, concentrated and analyzed by agar gel electrophoresis at pH 5.5.

*On Military Leave

Major Findings: Inasmuch as the backcross used was a double backcross, the proportion of recombination in the gametes could be estimated directly from the proportion of recombinant phenotypes among the offspring. In a study involving the electrophoretic phenotyping of urines from 121 mice the three recombination proportions found were Pintail - brown, 0.041; brown - Mup-a, 0.066; Pintail - Mup-a, 0.107. Thus the order of these loci on linkage group VIII is Pintail - brown - Mup-a, and the distances between successive loci determined from this study are 4.1 units and 6.6 units, respectively. The former value agrees well with the published figure for the distance between the Pintail and brown loci, namely 5.0 units.

Significance to Bio-Medical Research and the Program of the Division: The control program of the Laboratory continually requires information regarding the characteristics of proteins and methods used in studying them. The basic research program described above continues to provide the professional staff with a constantly updated pool of fundamental information (including research methodology) necessary in evaluating new products and tests and in discussions with manufactuers.

Proposed Course of Project: This is a continuing project.

Honors and Awards: None

Publications: Finlayson, J. S., Mushinski, J. F., Hudson, D. M., and Potter, M.: Components of the major urinary protein complex of inbred mice: separation and peptide mapping. Biochem. Genet. 2:137-140, Sept. 1968.

LCA

Annual Report of the
Laboratory of Control Activities
Division of Biologics Standards
July 1, 1968 through June 30, 1969

I. Mission

The Laboratory of Control Activities has prime responsibility within the Division for performing tests on biological products to determine their conformance to standards of safety, purity and potency. The Laboratory is composed of four sections: Analytical Chemistry, Control Test, Pyrogen Test and Reference Standards.

The Laboratory has the following functions:

(a) To conduct chemical, sterility, general safety, pyrogen, residual moisture and potency tests on biological products submitted for release or in support of license applications. Of all the various licensed products, the number tested by the Laboratory is 270 for sterility, 180 for safety, 90 for potency, 80 for chemical, 40 for pyrogen and 20 for residual moisture. Testing is accompanied by review of the manufacturers' protocols with respect to the tests performed by the Laboratory. Research is conducted on the development and modification of control tests on biological products.

(b) To develop, maintain and distribute material biological standards, references, control materials and reagents necessary for the testing of products to determine compliance with standards. A culture collection is also maintained to support the technical effort of the Division as well as to support control activities as they affect the licensed manufacturers.

(c) To maintain under review published standards, minimum requirements, technical memoranda and other guidance issued by the Division to manufacturers concerning licensable biological products, to develop or propose revisions in regulations when indicated, and to prepare technical specifications and other regulatory procedures for new products as they are developed.

(d) To maintain a close liaison and working relationship with other laboratories of the Division and where indicated with the other Institutes of N.I.H. as well as other outside agencies to insure a continuous flow of knowledge and technical information deemed vital for the proper exercise of control measures of both those products already licensed and new products being developed for license.

(e) To participate in and perform annual inspections of designated license establishments in accordance with specified procedures and specific objectives as prescribed by law.

(f) To participate in and give technical support to investigations performed by the Division in connection with Public Health Service Act.

(g) To participate in the review of applications and reports of manufacturers and their products to determine eligibility for license.

(h) To participate in and give technical support to personnel training programs organized within the Division of Biologics Standards.

II. Product Testing, Protocol Review, Records

Individual lots of licensed products are controlled by the Division through a system of evaluation and release utilizing data submitted by the manufacturer and that developed by the testing program of the Laboratory of Control Activities and other testing areas of the Division. All testing is directed toward the objective of assuring that products meet prescribed standards of safety, purity and potency.

Included in the purity testing of biological products are tests for pyrogenicity, residual moisture and specific chemicals, which also serve as means for evaluating and controlling the safety and stability of such products.

The types of products tested by the Laboratory include antitoxins and therapeutic immune serums, blood and blood derivatives, bacterial vaccines, toxins and toxoids, multiple antigens, viral and rickettsial vaccines, diagnostic substances for dermal tests, antivenins, venoms, bacterial enzymes and allergenic extracts.

In addition to the control testing of biological products within the Division, the Pyrogen Test Section of the Laboratory of Control Activities provides regular cooperative testing services to other N.I.H. facilities such as the Clinical Center Pharmacy, Clinical Center Blood Bank, Media Department, National Institute of Allergy and Infectious Diseases, and the National Cancer Institute; and the Control Test Section performs sterility tests for the Clinical Center Pharmacy and Blood Bank.

A summary of 10,612 tests performed by the laboratory during a 12 month period (April 1, 1968 through March 31, 1969) in making the determination that products meet prescribed standards is shown in Table 1.

III. Material Standards

An integral part of the system of manufacture and control of biological products is the application and use of material standards. By the use of such standards potency of a product can be determined and standardized so as to give increased assurance of consistency from lot to lot. It is the function of the standards program of the laboratory to provide these standards and reference preparations so that the control program of the Division as well as manufacturers and others engaged in testing may have available the tools to calibrate and evaluate products. It is largely through use of these preparations that those portions of the biologics control regulations relating to potency can be applied.

In order to maintain an adequate supply of material references and standards it was necessary to prepare and calibrate during the past year the following lots of materials: 13 antitoxins, 1 serum, 2 vaccines, 2 viruses, and 2 skin test antigens. In addition to these materials prepared by the laboratory the following lots of materials were purchased from commercial sources and finished by the laboratory: 2 antitoxin, 1 serum, and 6 vaccines. A total of 1,058 tests was required to complete a satisfactory standardization of these materials and maintain surveillance of the stability of all preparations. These tests included such procedures as flocculation reactions, animal protection tests, animal titrations, neutralizations and assorted *in vivo* and *in vitro* tests on specific specialized materials.

The technique for freeze-drying is a key procedure applied in the preparation of standards and reference materials whenever possible in order to increase assurance of stability over long periods of storage. The following numbers of ampules of various preparations were dried during the year.

Cultures	534	ampules
Serums	3,196	"
Vaccines	1,900	"
Viruses	1,461	"
Total	7,091	ampules

The following standard and reference preparations, consisting of a total of 988 separate shipments were distributed to manufacturers, P.H.S. and state health laboratories, universities, foreign manufacturers holding U. S. licenses and qualified foreign laboratories (primarily through the World Health Organization).

Antitoxins	483
Serums	4,323
Vaccines	6,226
Toxins	2,110
Bacterial & Viral Cultures	348
Allergens	676
Viruses	1,849
Opacity Standard	45
Venoms and Antivenins	8
Miscellaneous	4
Total	16,072

A culture collection of etiological agents, i.e. viral and bacterial organisms, used in the manufacturing and testing of biological products, is maintained. Approximately 100 different standards, references, and related control preparations are maintained for distribution.

IV. Related Activities

In addition to the effort outlined above, the laboratory staff also participated during the year in a number of ancillary activities directly related to control which accounted for a substantial segment of its working time.

(a) Members of the professional staff of the laboratory participate as members of DBS committees appointed to review, evaluate and recommend appropriate action with respect to new or revised product and establishment license applications submitted to the Division. Such review encompasses detailed scrutiny of manufacturer's production protocols for scientific accuracy and conformance to prescribed standards, testing of the products, inspection of manufacturing facilities, preparation of reports, and final recommendations. Laboratory personnel were members of 15 such committees during the year.

(b) The laboratory staff participated in 25 of the on-site inspections required to be performed annually on all licensed establishments. Approximately 28 man days of work were consumed during the year in this effort.

(c) Members of the staff participated in drafting and revising regulations and additional standards. Redrafts of technical recommendations for the manufacture of Smallpox Vaccine, Measles-Smallpox Vaccine, Typhoid Vaccine, and Tuberculin were prepared. Drafts of recommendations for manufacture of Rubella Vaccine were prepared.

V. Collaborative Studies with the World Health Organization

The Laboratory of Control Activities works closely with the biological standardization program of the World Health Organization. During the year it has participated in collaborative studies on the Proposed Second International Standard for Tetanus Antitoxin, the Proposed International Reference Preparation for Cholera Vaccine and the Proposed International Plain and Adsorbed Tetanus and Diphtheria Toxoid Preparations.

Periodically the International Reference Preparation for Rabies Vaccine (prepared by the Laboratory of Control Activities in 1963) is tested for potency by the laboratory in order to evaluate its stability. The Chief of the Laboratory served as a consultant to the World Health Organization in Geneva, Switzerland, and participated in the preparation of a draft of the International Minimum Requirements for Snake Antivenins.

VI. Research

While the primary function of the Laboratory of Control Activities is concerned with various aspects of controlling the safety, purity and potency of biological products, a substantial research program is conducted in order to provide a sound basis for the testing program and selection of preparations for use as official standards and references. The following are summaries of some of the research conducted by laboratory personnel during this fiscal year.

(a) Standardization of Coccidioidin: Studies have been initiated to develop a satisfactory laboratory assay for coccidioidin and to develop an improved reference preparation. Preliminary data indicate the feasibility of quantitative measurements of skin reactivity to antigen in sensitized guinea pigs.

(b) Clostridium botulinum Toxin and Antitoxin: In the study of the relationship of nutrition to biochemical structure of toxins from various types, several toxin preparations have been produced and a number of laboratory techniques modified to be more directly applicable to the objectives of this study. Work is progressing at a constantly excellerating rate.

(c) Coral Snake Venom and Antivenin: Work has continued on the comparison of electrophoretic patterns of venom from several species and subspecies of coral snakes. A manuscript has been prepared for submission for publication. Comparisons of homologous and heterologous venom neutralizing activity of several hyperimmune sera and antivenins are in progress.

(d) Opacity Standard: The U.S. Opacity Standard used in the standardization of cell concentrations of bacterial vaccines and of bacterial challenge suspensions used in potency tests is composed of finely divided glass particles suspended in water. During use and storage the opacity of the standard gradually lessens due to changes in particle size and adherence of particles to the walls of the container. Another undesirable feature is the difficulty of reproducing exactly the properties from lot to lot. For example the present international preparation differs from all previous international preparations as well as from the U. S. Standard. This is causing serious problems in laboratories throughout the world. It has been reported that a stable opacity preparation can be prepared by suspending finely divided titanium dioxide in a solid resin. We have initiated this project to determine if such a preparation is feasible for use as the U. S. Opacity Standard. In addition the use of finely divided glass in resin is being studied.

(e) Smallpox Vaccine: Studies associated with problems in the control of smallpox vaccine have been directed toward dried smallpox vaccine intended for administration by the jet gun. Because a portion of the vaccine is injected below the skin by the jet gun, it has been considered advisable to require that smallpox vaccine intended for jet injection be free of viable bacteria. Present vaccine prepared in the skin of calves is difficult to prepare free of viable bacteria. When present in relatively small numbers in smallpox vaccine, bacteria are difficult to detect. Therefore, studies have been initiated to devise sensitive techniques for determining the level of both viable aerobic and anaerobic bacteria in smallpox vaccine. These tests will then be applied to the control testing of vaccine.

Data are being collected using the Reference Smallpox Vaccine to estimate the variability of titrations on the chorioallantoic membrane versus in monkey kidney tissue cell culture. The correlation of these assays with skin reactivity is under investigation.

(f) U.S. Standard Tetanus Antitoxin: The supply of the dried master used in the preparation of the U. S. Standard Antitoxin is nearly depleted. This dried preparation was adopted in 1907 and has been in use since that date. Five lots of equine tetanus antitoxin and one lot of tetanus immune globulin (human) are being studied in order to select one having suitable properties for use as a replacement for the master preparation. Work to date indicates

that avidity of these preparations can be changed depending upon the type of toxin used.

All antitoxins tested continued to neutralize more toxin up to 48 hours after preparation of the toxin-antitoxin mixtures. Toxin solutions used in these studies did not begin to loose toxicity until after 48 hours. A glycerinated solution at 5 units/ml. (the strength at which the Standard is distributed) of one of the candidate lots was considerably less stable than solutions prepared from the other lots. This lot lost 20% in four months and 35% in six months of storage at 37°C. By 20 months this lot has lost 50% of its activity. Solutions of all lots were stable at 4°C. for at least 31 months. In addition this study and testing performed in the laboratory on lots submitted for release indicates that tetanus immune globulin (human) differs from tetanus antitoxin (equine) in the nature of the toxin-antitoxin neutralization reaction which occurs. Studies are in progress to investigate these differences. One obvious difference is in the type of antigen used to produce the antibodies. In the manufacture of tetanus antitoxin horses are hyperimmunized with relatively crude toxin following preliminary doses of toxoid. For tetanus immune globulin humans are hyperimmunized with toxoid of relatively high purity.

(g) Malayan Pit Viper Venom: It has been reported that a fraction of the venom from the Malayan pit viper (Agkistrodom rhodostoma) possesses anti-coagulant and thrombolytic activity. A few clinical studies have been reported as being encouraging. On the basis of these reports a study of the venom has been undertaken in order to provide basic information for eventual evaluation of license applications and control of the product. Three fractions have been prepared and are being studied.

TABLE 1

Samples Tested	Number of Tests Performed				
	Sterility	Safety	Potency	Purity	Total
Products for Release	3,988	1,120	905	1,199	7,212
Inspection	193	0	5	52	250
Cooperative Service	1,616	6	7	415	2,044
Reference and Standardization	463	0	582	13	1,058
Complaint	18	8	2	20	48
Totals	6,278	1,134	1,501	1,699	10,612

Serial No. DBS 29
1. Control Activities
2. Analytical Chemistry
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Development of methods for the chemical analysis of biological products.

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: Harry P. Marshall
J. Richard Miller
Clarence A. Robinson
Joseph W. West

Cooperating Units: None

Man Years

Total: 4.25
Professional: 2.25
Others: 2.0

Project Description:

Objectives: To develop new methods of chemical analysis of biological products or to modify existing methods, thus facilitating the study of chemical problems in the control of and research on biologicals.

Methods Employed: Standard methods of chemical analysis are used, evaluated, or modified as required for the material being analyzed, and new methods are developed.

Major Findings: 1) Work has continued on the evaluation of a method developed in the Analytical Chemistry Section to determine the mercurial preservative thimerosal (Merthiolate, Lilly) in biologicals. A collaborative study is now under way with a manufacturer of biologicals to analyze samples of biologicals by this method and by a traditional wet chemical method. The results have been in close agreement for most products tested so far. Some improvements in equipment only now becoming commercially available promise to make the method even more sensitive, precise, accurate, and rapid.

2) A program is now under way to analyze dried biologicals by both the electrolytic method and the traditional gravimetric method and correlate the results obtained by both methods.

3) Work has been initiated on developing a capability for performing quantitative assays of L-asparaginase as a control test.

4) A method to measure alum and aluminum phosphate in biologicals was developed in LCA. The results appear promising, but are still being evaluated.

5) The potential applications of glass powders of controlled pore size as matrices for steric exclusion chromatography of biological substances is being explored.

6) A collaborative study with Laboratory of Bacterial Products on the esterase activity of Clostridium tetani has been undertaken. Standard methods of gas chromatography are supplemented by specialized techniques for extraction and methylation of free and bound fatty acids from Arlacel A which has been treated with crude culture filtrate containing esterases. Knowledge of the interaction of Arlacel A and some antigens may be useful in determining the relationship of esterases to the adverse reactions of antigens in oil.

7) A collaborative study with Laboratory of Bacterial Products on the determination of acetyl groups in antigens of Escherichia coli has been undertaken using gas chromatography and special techniques for extraction and methylation of acetyl groups.

Significance to Bio-medical Research and the Program of the Division: The significance of this work lies in providing increased knowledge of the chemical composition of biologicals which can be of value in research, and in surveillance of these products for the protection of the public.

Proposed Course of Project: This project will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 36
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Standardization of Coccidioidin

Previous Serial Number: Same

Principal Investigator: Vernon J. Fuller, Ph.D.

Other Investigators: Sotiros D. Chaparas, Ph.D. (LBP)
Ruben Marquina

Cooperating Units: Laboratory of Bacterial Products

Man Years

Total: 0.5
Professional: 0.25
Other: 0.25

Project Description:

Objectives: To develop suitable laboratory procedures and reference preparations for use in the potency testing of coccidioidin using DBS Reference Coccidioidin, Lot 64D2.5.

Methods Employed: Guinea pigs were sensitized with coccidioidin in various combinations of spores and with or without Freunds adjuvant.

Major Findings: Animals are being sensitized and only a preliminary test of reactivity has been undertaken.

Significance to Bio-medical Research and the Program of the Division: It is anticipated that the new test procedure will have application in the regulation, control, and standardization of commercial coccidioidin preparations.

Proposed Course of Project: Studies to be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 143
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: *Bacteroides vulgatus* for Testing Fluid Thioglycollate Medium

Previous Serial Number: Same

Principal Investigator: Jane F. Farber

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Project Completed.

Publications: Farber, J.F., and Seligmann Jr., E.B.: *Bacteroides vulgatus*, a Nonsporeforming Anaerobe for Testing Fluid Thioglycollate Medium. *Applied Microbiology* 16(7): 1968.

Serial No. DBS 144
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Clostridium botulinum Toxin and Antitoxin

Previous Serial Number: Same

Principal Investigator: Lee J. Stevan, M.S.

Other Investigators: Edward B. Seligmann Jr., Ph.D.
Robert E. Grubbs, Jr.

Cooperating Units: None

Man Years

Total: 0.5
Professional: 0.25
Other: 0.25

Project Description:

Objectives: To establish the relationship of various nutritional components of the growth medium to the biochemical structure of toxins of various strains of Clostridium botulinum types A, B, C, and E.

Methods Employed: Techniques of immunochemistry, fractionation procedures, biochemistry and bacteriology.

Major Findings: Clostridium botulinum types A and B toxins have been produced and tested with the reference antitoxin. Currently strains of each type are being grown under various conditions. Toxin potency is then tested and culture filtrates of these strains will be analyzed by acrylamide disc electrophoresis.

Significance to Bio-medical Research and the Program of the Division: Potency testing of botulism antitoxins has been unsatisfactory primarily due to inadequate test toxins. A better understanding of the nature of botulinum toxins will lead to more satisfactory test procedures and possibly a better understanding of the mechanism of action of both the toxin and the antitoxin.

Proposed Course of Action: The work is in its initial stages. It will be expanded considerably during the coming year.

Honors and Awards: None

Publications: None

Serial No. DBS 145
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Coral Snake Venom and Antivenin

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: Lee J. Stevan, M.S.
William H. Berkeley
John H. Dawson

Cooperating Units: None

Man Years

Total: 1.25
Professional: 0.5
Other: 0.75

Project Description:

Objectives: Establishment of standards for coral snake antivenin. Study of the immunogenic properties of coral snake venoms from various species of snakes. Electrophoretic studies of coral snake venoms.

Methods Employed: Hyperimmunization of various species of animals. Venom neutralization tests. Immunoelectrophoresis, agar gel diffusion, acrylamide disc electrophoresis and agar gel electrophoresis.

Major Findings: Antisera have been prepared in rabbits, goats, ponies and burros against venom from a number of species of coral snake. Cross-neutralization tests indicate that two of these species have venoms which cannot be neutralized by anything other than homologous antiserum. Electrophoretic patterns indicate that purity and species identity of coral snake venoms may be determined by use of these techniques.

Significance to Bio-medical Research and the Program of the Division: As an outcome of work performed in the Laboratory, Wyeth Laboratories was licensed in September 1967 for Antivenin (Micrurus fulvius) which is effective against the venom of all coral snakes found in the United States except for Micruroides euryxanthus, the Arizona coral snake. Studies on cross-neutralization of venom from various species of coral snake are leading

to an understanding of the extent of usefulness of coral snake antivenins for treatment of bites from coral snakes found throughout North, Central and South America.

Proposed Course of Project: Completion of work in progress on cross-neutralization and immunochemical properties of venoms from various species. Completion of work in progress on the *in vivo* effectiveness of antivenins in treating envenomation.

Honors and Awards: None

Publications: None

Serial No. DBS 146
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Standardization of Reference Diphtheria Antitoxin for Flocculation Test

Previous Serial Number: Same

Principal Investigator: Jane F. Farber

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: None

Man Years

Total: 0.75
Professional: 0.5
Other: 0.25

Project Description:

Objectives: To select a suitable diphtheria antitoxin to replace the present Reference Diphtheria Antitoxin for Flocculation Test upon depletion and to establish Lf and L+ values equivalent to the present reference.

Methods Employed: Candidate antitoxins and the reference antitoxin were flocculated against the control toxin by both the Ramon and Dean-Webb methods to determine the Lf unitage and the Kf value. The L+ unitage of the antitoxins were established by guinea pig neutralization tests.

Major Findings: The diphtheria antitoxin that possessed the most suitable characteristics was selected from four candidate sera. The Lf unitage was established based on comparison tests with the reference antitoxin. A Kf value similar to that of the reference was one of the criteria used for selection. By comparing the neutralization capacity of the candidate antitoxin to that of the U. S. Standard Diphtheria Antitoxin, an L+ value was established. After vialing and freeze-drying the candidate antitoxin, the precise dilution factor for reconstitution was determined that yielded an Lf value equivalent to the present reference. The new preparation has been distributed to manufacturers for their approval and has been adopted for use.

Significance to Bio-medical Research and the Program of the Division: Reference Diphtheria Antitoxin for Flocculation Test provides the Division, biologic manufacturers and research workers in the field with a rapid means of measuring the activity of diphtheria antitoxins, toxins and toxoids in the form of final products and also during the manufacturing process.

Proposed Course of Project: As the candidate antitoxin has been found to be satisfactory for use as a new reference, the project is completed.

Honors and Awards: None

Publications: None

Serial No. DBS 147
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Development of an Improved Opacity Standard

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: Jane F. Farber
Harry P. Marshall

Cooperating Units: None

Man Years

Total: 0.25
Professional: 0.25
Other: 0

Project Description:

Objectives: 1) To prepare a stable solid opacity standard by suspending finely divided particles in a resin.

2) To standardize this preparation in terms of the current opacity standard which is an aqueous suspension of glass particles.

3) To determine the practicality of distributing a solid standard for use by manufacturers.

Methods Employed: Varying concentrations of titanium dioxide or glass particles are evenly suspended in viscous melted resin and allowed to harden in selected tubes. The concentration having the same light transmission as the U.S. Opacity Standard is then selected.

Major Findings: 1) Tubes of resin containing evenly suspended particles of titanium dioxide and having the same light transmission properties as the U.S. Opacity Standard have a different opacity when compared by means of the unaided eye.

2) Evaluation of a suspension of glass particles in resin is now in progress.

Significance to Bio-medical Research and the Program of the Division: The U.S. Opacity Standard is used to standardize the cell concentrations of bacterial vaccines and to achieve lot to lot consistency. The bacterial cell concentration of challenge doses used in potency tests are also adjusted with this standard. Because of the relative instability of the current aqueous suspension of glass particles a new lot must be prepared and distributed every few years. It is extremely difficult to reproduce the exact properties of a suspension of finely divided glass particles. Differences among lots and changes in a lot during use can lead to variation among lots of vaccine. As an example of the problems created by different lots of suspended glass opacity preparations the current lot of international reference differs significantly from any previous U.S. or international preparation and laboratories throughout the world are having difficulties in using it in the preparation of vaccines. Use of a permanent type of opacity standard such as a suspension of particles in a viscous resin or a solid plastic material of desired opacity would resolve many of these problems.

Proposed Course of Project: To prepare resin suspensions of particulate matter to determine the most suitable combination for use as the U.S. Opacity Standard. The possibility of using a solid plastic material of desired opacity is also being investigated. A sufficiently large quantity of the selected material will then be prepared for distribution.

Honors and Awards: None

Publications: None

Serial No. DBS 148
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Development of Sterility Test Procedures for Testing Dried Smallpox Vaccine for Jet Gun Injection

Previous Serial Number: Same

Principal Investigator: Vernon J. Fuller, Ph.D.

Other Investigator: Ruben Marquina

Man Years

Total: 0.5
Professional: 0.25
Other: 0.25

Project Description:

Objectives: To determine the incidence of contamination of commercial lots of dried smallpox vaccine for jet gun injection.

Methods Employed: Preliminary sterility testing with varying doses was carried out by the pour plate and test tube methods. The tube method was selected as the method of choice since the rate of contamination obtained by either method was essentially the same and because tests could be carried out more expeditiously.

Major Findings: Sterility testing of 30 vaccine lots (500 dose and 100 dose packages) showed that no lot yielded more than three positive thioglycollate tubes per each of 300 doses tested. These results are in agreement with those obtained by the manufacturer. Four known highly contaminated lots were tested in a similar manner. The DBS and manufacturer test results indicated a high order of contamination, 9-11 positive thioglycollate tubes, per each of 300 doses tested.

These findings indicate that the test method is sufficiently sensitive to detect minimal contamination of dried smallpox vaccine for jet gun injection when a minimum of ten doses of vaccine are tested.

Significance to Bio-medical Research and the Program of the Division: A method for detecting minimal contamination of smallpox vaccine (JGI) and supporting data are now available.

Proposed Course of Project: This study has terminated.

Honors and Awards: None

Publications: None

Serial No. DBS 150
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Replacement of the U. S. Standard Tetanus Antitoxin

Previous Serial Number: Same

Principal Investigator: Jane F. Farber

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: None

Man Years

Total: 0.5
Professional: 0.5
Other: 0

Project Description:

Objectives: To select a new lot of antitoxin that will prove suitable for use as a master dried U. S. Standard Tetanus Antitoxin, the supply of which is nearly depleted.

Methods Employed: The protective unitage is determined by comparing the toxin neutralizing capacity of the antitoxins with that of the standard antitoxin in mice and guinea pigs. Characteristics of the antitoxins such as avidity and heat stability are determined by the following methods using two different toxins: 1) Comparison of the L+ values of the antitoxins obtained at test levels of L+/10, L+/100 and L+/1000 in mice.

2) Stability of the toxin-antitoxin mixture after different incubation periods at room temperature.

3) Comparison of the L+ values of glycerinated antitoxins stored at 37°C. for lengthening periods of time.

Major Findings: 1) Avidity: The L+ values of all the equine antitoxins either increased or decreased to the same degree as the dilution factor of the test increased (L+/100 L+/1000). The increase or decrease of the L+ value was solely dependent upon the toxin used.

2) Stability of the toxin-antitoxin mixture: All the antitoxins increased in neutralizing unitage as the incubation period was lengthened

from 15 minutes to 48 hours after which time the LD_{50} of the toxin was no longer stable. This characteristic of the antitoxins was observed when tested against either toxin.

3) Decrease in unitage of the glycerinated antitoxins stored at 37°C.:
Lot C after 30 months decreased 53%
Lot F after 31 months decreased 52%
Lot E after 20 months decreased 55%

4) A rabbit antitoxin that was prepared and tested with a homologous toxin demonstrated similar characteristics as the heterologous test system in the initial tests.

Significance to Bio-medical Research and the Program of the Division: This study will aid in the selection of an antitoxin possessing the most suitable characteristics for use as the official U. S. Standard Tetanus Antitoxin.

Proposed Course of Project: A series of glycerinated lots of the candidate antitoxins have been prepared of varying concentrations. The exact neutralizing unitage of these lots will be established to determine which concentration most closely equals the unitage of the standard antitoxin. The characteristics of tetanus immune globulin (human) and the homologous test system will be further studied in an attempt to clarify the problems encountered in the animal neutralization tests.

Honors and Awards: None

Publications: None

Serial No. DBS 153
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Development of a Reference Typhus Vaccine and an Improved Potency Test

Previous Serial Number: Same

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigators: Otis L. Green
Harry T. Aylor
Elmer C. Russell

Cooperating Units: None

Man Years

Total: 0.1
Professional: 0.03
Other: 0.07

Project Description:

Objectives: Establishment of a suitable Reference Typhus Vaccine preparation. Development of an improved potency test for typhus vaccine.

Methods Employed: Standard potency test described in the Minimum Requirements for Typhus Vaccine and a variety of modifications including antigen extinction type tests.

Major Findings: This project is being continued at a rate subject to the availability of sufficient guinea pigs and higher priority control testing.

Significance to Bio-medical Research and the Program of the Division: The effectiveness of present typhus vaccine has been questioned by the scientific community concerned with the use of typhus vaccine in the armed services. A reference vaccine would aid in assuring more consistency among production lots. In addition a more sensitive potency test is required in order to detect differences among lots more reliably. The present potency test consists of two immunizing doses which tends to mask differences in vaccine activity.

Proposed Course of Project: Under contract, typhus vaccine at several two-fold gradations in titer is being obtained for use in correlating laboratory assay procedures and clinical response.

Honors and Awards: None

Publications: None

Serial No. DBS 154
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Bactericidal Activity of Wasp Extract

Previous Serial Number: Same

Principal Investigators: Edward B. Seligmann Jr., Ph.D.
Jane F. Farber

Other Investigator: Dr. Allen W. Benton, Department of Entomology, Pennsylvania State University

Cooperating Units: Department of Entomology, Pennsylvania State University

Man Years

Total: 0.1
Professional: 0.1
Other: 0

Project Description:

Objectives: 1) To detect the presence of and evaluate the factors found in wasps and hornets which inhibit growth of bacteria and fungi.

2) To isolate and identify the inhibiting agent(s).

Methods Employed: Extracts prepared from wasps and sterilized by filtration are tested for inhibitory effects on the growth of a variety of microorganisms.

Major Findings: Extracts prepared from the bodies of White Faced Hornet queens and from Yellow Jacket queens demonstrated inhibition upon the growth of several Gram negative bacteria and spore-forming anaerobes but failed to inhibit the growth of a fungus tested.

Significance to Bio-medical Research and the Program of the Division: This project originated out of an observation that bodies of wasp and hornet queens failed to deteriorate in a freezer after a power failure. The bodies of other insects stored in the same freezer decomposed. The queens normally overwinter either in the ground or in suspended nests. It is reasonable to assume that they may be resistant to invasion by soil microorganisms. Substances inhibitory to certain classes of bacteria found in the soil and to

certain fungi could be of significance in the treatment of diseases and injuries of man.

Proposed Course of Project: An attempt will be made to isolate and identify the inhibitory agent(s).

Honors and Awards: None

Publications: None

Serial No. DBS 155
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: RIF-free Yellow Fever Virus Seed

Previous Serial Number: Same

Principal Investigator: Jane F. Farber

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: Laboratory of Virology & Rickettsiology
Laboratory of Pathology

Man Years

Total: 0.35
Professional: 0.1
Other: 0.25

Project Description:

Objectives: To compare the safety and antigenicity of the RIF-free primary and secondary yellow fever seed viruses to that of the current primary and secondary seed viruses.

Methods Employed: The characteristics of the seed viruses were compared by the methods described in WHO Technical Report Series No. 179, 1959 whereby a group of monkeys were immunized with the viruses. The level of viscerotropism of each virus was determined by mouse titration of the circulating virus present in the monkey blood on specific post immunization days. Within 30 days after immunization, the antibody level of the pre and post monkey sera was demonstrated by neutralization of a challenge virus in mice. The appearance of clinical manifestations of encephalitis in monkeys indicated the degree of neurotropism of the virus.

Major Findings: Laboratory phases have been completed and results are being submitted for publication.

Significance to Bio-medical Research and the Program of the Division: Before use of a RIF-free yellow fever seed virus in the production of vaccine it was essential to demonstrate that manipulation of the virus necessary to free it of RIF had not altered its characteristics of safety and antigenicity.

Proposed Course of Project: Clinical studies are planned to be initiated at an early date.

Honors and Awards: None

Publications: None

Serial No. DBS 168
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Malayan Pit Viper Venom

Previous Serial Number: None

Principle Investigators: Lee J. Stevan, M.S.
D.L. Aronson, M.D. (LBBP)

Other Investigator: Edward B. Seligmann Jr., Ph.D.

Cooperating Units: Laboratory of Blood and Blood Products

Man Years

Total: 0.5
Professional: 0.25
Other: 0.25

Project Description:

Objectives: To describe and evaluate the anti-coagulant and thrombolytic activity of Malayan pit viper (Agkistrodon rhodostoma) venom.

Methods Employed: Chromatography and electrophoresis, necessary tests used to evaluate the various venom fractions such as fibrinolysis, hemorrhagic activity and in vitro clotting of fibrinogen.

Major Findings: Column chromatography of the venom has yielded three protein fractions:

Fraction #1 (F) with fibronolytic activity
Fraction #2 (H) with hemorrhagic activity
Fraction #3 (T) with thrombin-like activity

These three fractions are in the process of being characterized and studied. Tests for each fraction have been developed and each fraction is being evaluated for its anti-coagulant, thrombolytic, and lethal activities as well as for possible harmful effects.

It is postulated that fraction #3 is identical to Arvin, a commercial product made in England from this venom, but a direct comparison has not yet been made. Laboratory results to date are compatible with a mechanism of action for Arvin which proposes that it is an activator for Factor XIII but more work is required.

Significance to Bio-medical Research and the Program of the Division: This investigation will lead to an understanding of the anti-coagulant and thrombolytic effects of Malayan pit viper venom. This understanding will in turn help the Division evaluate the potency and purity of any new drugs derived from this venom.

Proposed Course of Action: Attempts will be made to differentiate the anti-coagulant effect from the thrombolytic effect of Malayan pit viper venom and to identify the protein fraction or fractions involved in each effect. The mechanism of action of the effects will also be investigated.

Honors and Awards: None

Publications: None

Serial No. DBS 169
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Comparison of Titrations in Monkey Kidney Tissue Cells and Chorioallantoic Membrane of Chick Embryos for the Potency Assay of Smallpox Vaccine

Previous Serial Number: None

Principal Investigator: Vernon J. Fuller, Ph.D.

Other Investigator: Ruben Marquina

Man Years

Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: To compare the potency of the U. S. Reference as determined by the chorioallantoic membrane method and the monkey kidney tissue cell (MKTC) method.

Methods Employed: The chorioallantoic membrane technique and MKTC method were employed in this study.

Major Findings: Initial results indicate that the two test systems yield potency values of the same magnitude. The U. S. Reference gave a mean titer of $10^{8.1}$ PFU/ml when tested by the CAM technique and $10^{8.4}$ PFU/ml when tested by the MKTC method.

Significance to Bio-medical Research and the Program of the Division: If the variability of the procedure can be brought under control, a third potency test method applicable to the standardization of smallpox vaccines may result from this study.

Proposed Course of Project: This study will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 170
1. Control Activities
2. Reference Standards
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Standardization of Reference Blood Group Substance A and B.

Previous Serial Number: None

Principal Investigator: Edward B. Seligmann Jr., Ph.D.

Other Investigator: Jane F. Farber

Cooperating Units: None

Man Years

Total: 0.25
Professional: 0.25
Other: 0

Project Description:

Objectives: 1) To select suitable blood group substances A and B to replace the present Reference Blood Group Substance A and B upon depletion.

2) To establish the unitage of the A and B substances compared to the current reference.

3) To modify the hemagglutination inhibition test procedure to increase its degree of accuracy and reproducibility.

Methods Employed: A more accurate and reproducible hemagglutination inhibition test procedure will be developed which will be used to compare candidate A and B substances with the present reference.

Major Findings: Work on this project has just been initiated. Highly purified lots of A substance and B substance have been obtained as candidate reference source material.

Significance to Bio-medical Research and the Program of the Division: Reference Blood Group Substance A and B is used by the Division, manufacturers, and research workers to determine the amount of residual A and B substances present in certain biological products such as toxoids, globulins and bacterial vaccines. The amount of contaminating substances

in such products must be kept at a minimal level to prevent the possibility of hyperimmunizing recipients of such products.

Proposed Course of Project: 1) Various proposed modifications of the hemagglutination inhibition test will be compared and if necessary further modified to establish the most reliable test system.

2) Characteristics of candidate A and B substances will be compared to those of the present reference to aid in the selection of a new reference.

3) After dissolving and combining the A and B substances in the desired concentrations the material will be vialed and dried. This preparation will then be distributed to the manufacturers and interested research workers for their review and eventual adoption if satisfactory.

Honors and Awards: None

Publications: None

LP

Annual Report of the Laboratory of Pathology
Division of Biologics Standards
July 1, 1968 through June 30, 1969

The Laboratory of Pathology has a continuing program to study the pathogenesis of infectious diseases using animal models. This program is designed to meet the overall needs of the Division in regard to the safety and efficacy of new biological products and also those which have been licensed for many years.

The program of testing the neurovirulence properties of Poliovirus Vaccine, Live, Oral, has centered on the completion of a collaborative study involving the control authorities in England and West Germany as well as the Laboratory of Pathology, DBS. The results indicate that all three laboratories can identify lots of vaccine of clear-cut increased neurovirulence but that technique of inoculation is most important in the study of vaccines of neurovirulence which is minimally increased.

This year, as last, with the extremely low level of poliomyelitis in the United States and in the world, the number of lots of vaccine submitted for testing was very small. However, the laboratory does perform neurovirulence tests on all of these lots at the present time. The importance of such continued testing was emphasized at a recent World Health Organization conference on Poliovirus Vaccine to which the Chief, Laboratory of Pathology, was a consultant. At this meeting it was stressed that extreme care must be exercised continuously in order to maintain the safety of the vaccine. (Project Number 41, p. 115)

Much of the effort of the laboratory is now being directed toward research in, and the development of, significant test systems for the newer attenuated viral vaccines and improvement of test systems for the older viral vaccines.

Yellow Fever Vaccine (17D strain) has been under study in collaboration with the Laboratory of Virology and Rickettsiology and the Laboratory of Control Activities. The neurovirulent properties of this virus have been found to be stable even when a virus of the avian leucosis complex has been removed from the seed virus pool. Since Yellow Fever is a disease that affects the central nervous system as well as the viscera, and since the vaccine has neurotropic properties for rhesus monkeys, control of the stability of this neutropism at a low level is essential for use of this new and more purified vaccine in man. The immunogenicity of this derivative of the primary seed strain and its stability are being investigated. (Project Number 112, p. 123)

In collaboration with the Laboratory of Viral Immunology, studies of wild and attenuated rubella virus both in pregnant and nonpregnant rhesus monkeys have been completed. The virus can infect these animals and multiply, although no clinical syndrome develops in either the adult or the fetus.

The safety tests for Rubella Virus Vaccine, Live, have been based on these studies and the Additional Standards promulgated. (Project Number 112, p. 123)

In collaboration with the Laboratory of Virology and Rickettsiology and Dr. Kendall Smith, studies of wild and "vaccine" strains of human adenovirus 4 are complete. Attempts to infect monkeys appear to be unsuccessful but the virus apparently does cause an inflammatory reaction when placed directly within the central nervous system. This reaction can be found whether crude or purified virus is used. (Project Number 112, p. 123)

The Section of Histopathology has expanded its activities in electron microscopy greatly and has developed excellent techniques. Studies of the ultrastructure of the central nervous system are designed to complement previous similar studies using light microscopy. (Project Number 45, p. 121) As the Section has perfected its techniques for ultrastructural studies, it has developed many collaborative programs. The Section of Histopathology has continued to perform clinical laboratory techniques and operates an automatic counter for assaying red and white blood cells. This has enabled the technical staff to perform such procedures with great efficiency.

Dr. Clark has expanded his studies of myelinization of the central nervous system of animals to include studies of demyelinating diseases, such as experimental allergic encephalomyelitis, and has initiated a study of congenital deformities of the central nervous system of animals. Such a baseline study is essential for subsequent evaluation of teratogenesis in relationship to the use of biological products. (Project Number 45, p. 121)

Studies by Dr. Clark, in collaboration with the Laboratory of Bacterial Products, have shown that pertussis vaccine has a specific adjuvant effect upon the induction of experimental allergic encephalomyelitis in the rhesus monkey. Attempts to induce experimental allergic encephalomyelitis in a number of inbred strains of mice have been unsuccessful. (Project Number 45, p. 121)

Further, Dr. Clark, in collaboration with the Surgical Neurology Branch, NINDS, has developed an experimental model for a debilitating abnormality of the central nervous system, namely, hydrocephalus. These studies will be expanded in order to evaluate the possible role of viral infections in the development of such an abnormality. (Project Number 45, p. 121)

The Section of Animal Testing, in addition to performing neurovirulence tests on all lots of Poliovirus Vaccine, Live, Oral, and selected lots of Poliomyelitis Vaccine, Measles Vaccine, Live; Mumps Vaccine, Live; and Rubella Virus Vaccine, Live, has maintained a colony of South American monkeys, namely, marmosets (Saguinus mystax). Recently, interest in this primate has increased greatly since it has been found to be susceptible to Rous sarcoma virus and has been thought possibly to be of use in the study of the pathogenesis of infectious hepatitis. Therefore, in October, 1967, the Animal Test Section obtained and established a colony of 86 marmosets. At present, there are 53 animals remaining. The problems of colony maintenance and nutrition are under control. Attempts to produce live young from the colony are being intensified

in the hope that a nucleus of laboratory bred marmosets will be available for further studies. A complete microbiological profile, as well as certain baseline values, has been established. Studies concerning the usefulness of this marmoset as an animal model for human hepatitis are just underway. (Project Number 133, p. 130)

The laboratory staff participate in a large program of collaborative research projects which are underway not only with other laboratories in the Division but with NCI, NIAID, NINDS, and NIMH. Some of these projects are specifically related to control activities of the Division and others have a tangential relationship. This collaborative research program is under the direction, supervision, and coordination of the Chief of the Laboratory.

Further, the Chief of the Laboratory has directed most of her own research efforts in the general field of the pathogenesis of infectious diseases with particular emphasis on oncogenic viruses. The pathogenesis of the particular tumors induced, the effect of altered immunologic mechanisms, and the interaction of these viruses on each other are all being studied. The inhibitory effect of one microbial agent on another both in regard to oncogenicity as well as infectivity is of particular interest. Thus, the inhibitory effect of adeno-associated viruses on the oncogenicity of adenovirus 12 in newborn hamsters is matched by the inhibitory effect of adenovirus 5 on the replication of certain viruses in hamster cell cultures. (Project Number 44, p. 117)

In addition, two members of the staff of the Contracts Operations office are engaged in research projects under the supervision of and in collaboration with the Chief of the Laboratory. One, Dr. Stanley Singer, is studying the basic interaction between microbial agents. He has found, as mentioned above, that certain viruses, such as adenovirus 5, inhibit the replication of other viruses, whereas, certain mycoplasmas enhance the replication of viruses. (Project Number 131, p. 128) The other, Dr. John Petricciani, has just begun a study of the effect of in utero infection on chromosomes. (Project Number 161, p. 135) Mr. Edward Fitzgerald, previously performing some studies under the direction of the Chief, Laboratory of Pathology, has transferred to the Laboratory of Control Activities in order to relate his studies more closely to certain control tests.

Collaborative (Contracts) Activities:

Dr. Amos E. Palmer of the Laboratory of Pathology was Project Officer for a contract set up to study the possible oncogenesis of biological products in newborn hamsters (PH43-67-1483). Since Dr. Palmer's departure, Dr. Stanley Singer, Mr. Edward Fitzgerald, and Mrs. Claire Cox have monitored this contract closely. The activities involving this contract have been closely supervised by the Chief of the Laboratory of Pathology who is reviewing the histologic material evolving from this contract.

Since many of the contracts which are underway have aspects relating to pathogenesis, the Chief of the Laboratory of Pathology works very closely with the Contracts Operations Staff and spends much time helping to plan and execute and to review progress reports of those contracts already in existence.

The Laboratory is cooperating in performing the pathologic examinations that are necessary for certain contract operations in which the contractor cannot do these examinations himself, namely, the contract on the effects of storage conditions on plastic blood containers (PH43-65-1046 and PH43-68-97). Histologic material from other contracts are under review particularly those involving the oil adjuvants (PH43-67-679).

Control Activities:

During the past year, the Laboratory has performed neurovirulence tests on 20 lots of Poliovirus Vaccine, Live, Oral, for release as well as tested the Reference Vaccine on eight occasions. Two lots of Poliomyelitis Vaccine and 12 lots of Measles Virus Vaccine have been tested. Five lots of Mumps Virus Vaccine have been tested. Four lots of experimental Rubella Virus Vaccine and six lots of such vaccine submitted in support of a license application for this product have been tested. A total of 1330 monkeys have been used for this purpose. The safety of these vaccines has been well controlled.

In addition, the Section on Animal Testing performed five potency tests for Poliomyelitis Vaccine using 60 monkeys. One hundred and fifty-five rhesus monkeys and 77 vervet monkeys were sacrificed in order to remove kidneys for the Cell Biology Section, LVR. Three hundred rabbits were similarly sacrificed. Forty monkeys were bled and held in quarantine for the Laboratory of Biophysics and Biochemistry.

The Histopathology Section produced 20,510 sections for light microscopy, 1400 sections for electron microscopic study, and performed 1100 complete blood counts.

The Chief of the Laboratory has served as Chairman of six license application review committees and on three other such committees. She has assisted in preparing regulatory standards for Smallpox Vaccine, Smallpox-Measles Vaccine, Rubella Virus Vaccine, Live, and revisions of the standards for Poliomyelitis Vaccine and Poliovirus Vaccine, Live, Oral. Protocols have been reviewed prior to release as follows: Poliovirus Vaccine, Live, Oral: 14; Poliomyelitis Vaccine: 3; Live Attenuated Measles Virus Vaccine: 266; Mumps Virus Vaccine, Live: 13. Each staff member has participated in the annual inspection program performing inspections at five establishments.

Serial No. DBS 41
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Developmental Studies of Neurovirulence Testing on Poliovirus Vaccine, Live, Oral, in Monkeys

Previous Serial Number: 41

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators: Ronald G. Clark, Ph.D., LP-DBS
George Rusten, LP-DBS

Cooperating Units: Serology Section, LVI, screens monkey serums for freedom from antibody to poliovirus.

Man Years:
Total: 5.0
Professional: 0.4
Other: 4.6

Project Description:

Objectives: To develop information concerning the reproducibility and sensitivity of neurovirulence tests of Live Poliovirus Vaccines and to refine techniques used in the test to assure safety of the vaccine.

Methods Employed: Monkeys were inoculated with vaccine or dilutions thereof either intrathalamically or intraspinally and observed for evidence of developing paralysis over a period of 17-18 days. The monkeys were then sacrificed and the central nervous system examined histologically for evidence of poliomyelitis. In addition, tissues were saved for virus isolation attempts. These studies were performed as outlined in the PHS Regulations for Live Poliovirus Vaccine, Live, Oral.

Major Findings: Five Type I or Type III coded vaccines were tested repeatedly both in the Laboratory and in two European control laboratories. The results completed have been analyzed and indicate that increased neurovirulence can be found by either intraspinal inoculation of cynomolgus monkeys or intrathalamic inoculation of rhesus monkeys. The latter method, when performed correctly, better discriminates levels of neurovirulence.

The code has been broken and the results indicate that all the laboratories were able to identify the one lot of vaccine selected because of previous evidence of increased neurovirulence.

In order to continue to ensure the safety of Poliovirus Vaccine, Live, Oral, as used in the United States, all lots of vaccine submitted for release have been tested. Six Type I lots and 10 Type 3 lots have been tested and found satisfactory. The Reference Vaccine has been tested four times.

Significance to Bio-Medical Research and the Program of the Division: These studies are designed to assure standardization of neurovirulence testing of poliovirus vaccines throughout the world, to assure the safety of such vaccines, and to improve testing procedures.

Proposed Course of Project: It is anticipated that collaborative studies to standardize safety testing of vaccines will be a continuing project. The present study is being prepared for publication.

Honors and Awards: Dr. Ruth L. Kirschstein served as a consultant to the World Health Organization on use of poliovirus vaccines, March 17-21, 1969.

Publications: None

Serial No. DBS 44
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Pathogenesis of Infectious Diseases

Previous Serial Number: 44

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators: Alan Rabson, M.D., NCI
Samuel Baron, M.D., NIAID
Elizabeth Peters, LP-DBS
Kendall Smith, Ph.D., LBB-DBS
K. K. Takemoto, Ph.D., NIAID
Harvey Ozer, M.D., NIAID
Hilton Levy, Ph.D., NIAID
Sotiros Chaparas, Ph.D., LBP-DBS
Carolyn Hardegree, M.D., LBP-DBS
John Petricciani, M.D., LP-DBS

Cooperating Units: Pathologic Anatomy Branch, NCI
Laboratory of Biophysics & Biochemistry, DBS
Laboratory of Biology of Viruses, NIAID
Laboratory of Viral Diseases, NIAID
Laboratory of Bacterial Products, DBS

Man Years:
Total: 7.0
Professional: 1.8
Other: 5.2

Project Description:

Objectives: Experimental infectious diseases are studied as to pathogenesis in the hope that this may further elucidate the pathogenesis of infectious diseases in man and lead to their prevention.

Methods Employed: Infectious agents and related substances or substances causing an immunologic response are injected into a variety of animals when newborn or adult, and gross autopsies and histologic examinations are performed at timed intervals so that the development of the disease process and the pathologic changes may be studied.

Major Findings: (1) (With Dr. Rabson and Mrs. Peters) Studies on the comparative oncogenic effects of polyoma virus strains, simian virus 40, and adenoviruses are continuing. The oncogenic potential of these viruses can be enhanced by decreasing the immunologic capability of these animals. The effect of various drugs and chemicals on the oncogenic potential and on the lethality of these viruses for various species of animals is under investigation.

(2) (With Dr. Rabson and Mrs. Peters) Studies of cultured cells from lymphomas of the "Burkitt" type and of viruses found by others to be associated with them are in progress. These cells have a limited ability to grow in heterologous hosts.

The pathogenesis of the Herpes group of viruses in newborn and adult hamsters, mice and rats after various routes of inoculation is being studied. Despite the association of "herpes-like particles" with the "Burkitt" type lymphoma, Herpes simplex had no effect on the lympho-reticular system. Attempts to alter its infectivity and decrease the immediate mortality are underway, using a variety of chemical and physical means. Other members of the Herpes group are being studied also.

(3) (With Dr. Rabson, Dr. Petricciani and Mrs. Peters) A viral induced rat sarcoma is being studied as to its growth characteristics in other species.

(4) (With Dr. Rabson) Studies of the ability of the oncogenic viruses to "transform" hamster cells and monkey cells in cell cultures are continuing. The cellular transformations have characteristics of malignancies and varying growth potential when inoculated into animals, depending on the species. Attempts to alter this growth potential by changing immune response and using other target organs are continuing.

(5) (With Drs. Takemoto and Ozer) Studies on the immunologic, oncogenic, and cytopathic effects of various SV40 plaque strains have been completed. Such strains have different oncogenic potentials both in vitro and in vivo.

(6) (With Dr. Hardegree) Studies of the pathologic reaction in muscles and ears of rabbits to mineral oil and Arlacel A (mannide monoleate) indicate that while each causes a granulomatous reaction, that caused by Arlacel A is much more severe. However, the free fatty acids (considered by some to be the toxic element in the Arlacel A) do not cause the same severe reaction that the Arlacel A does. Further studies of other adjuvants are in progress.

(7) (With Dr. Smith) The oncogenicity of adenovirus 12 for newborn hamsters is partially inhibited by the presence of adeno-associated virus (AAV). Thus, 60 per cent of newborn animals inoculated with adenovirus 12 alone developed tumors whereas only 19 percent of those inoculated with adenovirus 12 and AAV had tumors. The incubation period was also prolonged in those hamsters inoculated with adenovirus 12 and AAV. This model will be adapted to study other possible oncogenic agents.

(8) (With Dr. Chaparas) Studies of the development of antibody-producing cells in the lymph nodes of guinea pigs required the use of specialized stains. These studies are complicated by the natural flora of the animals which alters the production of antibodies. Inoculation of B.C.G. in adjuvant produces different reactions, depending on the sensitization state of the guinea pigs.

(9) (With Dr. Baron) A new synthetic double-stranded nucleic acid (polyinosinic, polycytidylic acid) appears to be a strong inducer of interferon both in vitro and in vivo. Since it is hoped that this substance may have much practical application, studies of its toxicity in a variety of laboratory animals are in progress.

(10) (With Dr. Levy) Studies as to the effect of this compound on viral induced tumors of hamsters are in progress.

Significance to Bio-Medical Research and the Program of the Division: These studies are designed to elucidate aspects of the pathogenesis of infectious diseases in animals so that this model may serve to help provide the means of prevention of such diseases in man.

Proposed Course of Project: It is expected that this program will expand in 1969-70.

Honors and Awards: Dr. Ruth L. Kirschstein appointed to the Contract Review Committee, Breast Cancer Task Force, N.C.I.

Publications:

Hardegree, M. C., and Kirschstein, R. L., The Toxicity of Free Fatty Acids and Arlacel A. Annals of Allergy, 26:259-268, 1968.

Kirschstein, Ruth L., Smith, Kendall O., and Peters, Elizabeth A., Inhibition of Adenovirus 12 Oncogenicity by Adeno-Associated Virus. Proc. Soc. Exp. Biol. and Med., 128:670-673, 1968.

Chaparas, Sotiros D., and Kirschstein, Ruth L., The Role of Delayed Hypersensitivity and Toxicity of BCG in the Development of Lesions at Sensitization Sites, Proc. Soc. Exp. Biol. and Med., 129:601-605, 1968.

Ozer, H. L., Takemoto, K. K., Kirschstein, R. L. and Axelrod, D., Immunochemical Characterization of Plaque Mutants of Simian Virus 40, J. of Virology, 3:17-24, 1968.

Singer, S. H., Kirschstein, R. L. and Baron, S., Viral Inhibition of Adenovirus 5 Treated Hamster Cells, Proc. Soc. Exp. Biol. and Med., 130:376-381, 1969.

Singer, Stanley H., Barile, Michael F., and Kirschstein, Ruth L., Enhanced Virus Yields and Decreased Interferon Production in Mycoplasma Infected Hamster Cells, Proc. Soc. Exp. Biol. and Med. (in press).

Singer, Stanley H., Kirschstein, Ruth L., and Barile, Michael F., Enhanced Vesicular Stomatitis Virus Yields from Mycoplasma Infected Hamster Cells, Nature (in press).

Serial No. DBS 45
1. Pathology
2. Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Histologic and Ultrastructural Development of the Central Nervous System and its Relationship to the Immunological Response

Previous Serial Number: 45

Principal Investigator: Ronald G. Clark, Ph.D.

Other Investigators: Philip McGrath, LP-DBS
Joyce Johnson, LP-DBS
Charles Manclark, Ph.D., LBP-DBS
Walter Stanley, Ph.D., LP-NIMH
Paul Albrecht, M.D., LVR-DBS
Thomas Milhorat, M.D., SN-NINDS
Sotiros Chaparas, Ph.D., LBP-DBS

Cooperating Units: Laboratory of Bacterial Products, DBS
Laboratory of Virology and Rickettsiology, DBS
Laboratory of Psychology, NIMH
Surgical Neurology Branch, NINDS

Man Years:
Total: 2.5
Professional: 1.5
Other: 1.0

Project Description:

Objectives: To ascertain the normal developmental processes in the nervous system of laboratory animals and to relate ultrastructural alterations of these developmental processes in experimental animals in response to immunologic stimulation.

Methods Employed: In utero and postnatal animals are sacrificed at timed intervals, and gross evaluation and histologic examinations are performed to determine the normal development of the central nervous system. The histologic evaluation involves both light and electron microscopy. Experimental animals are studied similarly, and microscopic as well as ultrastructural deviations from the normal are noted and evaluated.

Major Findings: (1) (With Dr. Manclark) It has been found that pertussis vaccine has an adjuvant effect in combination with complete Freund's adjuvant and CNS tissue in inducing experimental allergic encephalomyelitis (EAE) in the rhesus monkey.

(2) (With Dr. Stanley) A very high incidence of hydrocephalus was found to exist in at least three supposedly healthy beagle colonies. It is suspected that hydrocephalus is quite prevalent in beagles in general and probably is common in many species of dogs.

(3) (With Dr. Albrecht) Twenty-three strains of mice were injected with complete Freund's adjuvant and CNS tissue in order to find strains susceptible to allergic encephalomyelitis. None of the mouse lines inoculated demonstrated lesions of encephalitis.

(4) (With Dr. Milhorat and Mr. McGrath) Experimental obstructive hydrocephalus was produced in the rhesus monkey, representing the first such experimental model to be developed for the study of this disease in primates.

Significance to Bio-Medical Research and the Program of the Division: These studies are closely related to the problem of postvaccinal encephalitis and the production of vaccines in tissue from the central nervous system of animals and also may elucidate the causation of congenital abnormalities of the central nervous system.

Proposed Course of Project: The endeavor will continue with emphasis being placed on the study of ultrastructural changes in relation to immunological response.

Honors and Awards: Invited exhibit at the Centennial Meeting of the Cushing Society, Cleveland, Ohio, April 14-18, 1969, "Obstructive Hydrocephalus," Thomas Milhorat, M.D., Ronald G. Clark, Ph.D., and Giovanni Di Chiro, M.D.

Publications: None

Serial No. DBS 112
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Developmental Studies and Control Testing of Attenuated Live Virus Vaccines

Previous Serial Number: 112

Principal Investigator: Ruth L. Kirschstein, M.D.

Other Investigators: Ronald G. Clark, Ph.D., LP-DBS
Harry Meyer, M.D., LVI-DBS
Paul Parkman, M.D., LVI-DBS
Hope E. Hopps, LVI-DBS
Nicola Tauraso, M.D., LVR-DBS
Edward B. Seligmann, Ph.D., LCA-DBS
Kendall Smith, Ph.D., LBB-DBS
Frank Pedreira, M.D., LVR, NIAID

Cooperating Units: Laboratory of Viral Immunology
Laboratory of Virology and Rickettsiology
Laboratory of Biophysics and Biochemistry
Laboratory of Control Activities

Man Years:
Total: 3.0
Professional: 0.4
Other: 2.6

Project Description:

Objectives: To gain information concerning the pathogenesis of rubella virus, mumps virus, and adenovirus infections in monkeys so that safety tests for attenuated live virus vaccines can be developed and the safety of the vaccines assured.

Methods Employed: Rhesus monkeys are inoculated with both wild and attenuated virus preparations, using various routes of administration depending on the mode of action of the particular virus. Animals are observed for evidence of disease, and specimens of blood and various secretions are taken for evidence of virus multiplication. Uninoculated animals may be placed in cages with inoculated animals or in the same room in order

to study transmissibility of the wild or attenuated infection. At the end of the observation period which is usually 17-30 days, depending on the virus and the route of inoculation, all animals are bled to determine antibody levels. The monkeys are then sacrificed, and various organs are assayed for presence of virus. All organs from each animal are examined histologically for evidence of abnormality.

Major Findings: (1) (With LVR and LCA) Studies of Yellow Fever Vaccine (17D strain) after removal of the contaminating avian leucosis virus indicate that its neurovirulence has not been altered and that it is immunogenic for monkeys. This indicates that the vaccine has retained its desirable properties during the processing to remove the adventitious virus.

(2) (With LVI) Studies of the pathogenesis of rubella virus infection in monkeys are complete. To date, the virus has been found to multiply in the monkey but not to produce any pathologic alterations. On the basis of this study, the neurovirulence safety tests for rubella virus vaccine have been developed. Three experimental lots have been tested as well as six lots of rubella virus vaccine submitted in support of a license application for this product.

(3) (With LVI) Studies of the pathogenesis of mumps virus infection in monkeys indicate that these animals do develop neutralizing antibody after inoculation, but that no virus can be recovered from any tissues. No differences between a wild and an attenuated strain could be noted. Despite the lack of evidence of viral replication in the central nervous system, certain pathologic changes appear to occur consistently when the virus is inoculated intrathalamically. These lesions appear to be unrelated to neurovirulence of this virus for man. On the basis of these studies, the neurovirulence safety tests for mumps vaccine were developed.

(4) (With LVR) Attempts were made to infect rhesus monkeys with human adenoviruses and studies indicate that replication of the virus may occur occasionally. However, the rhesus monkey is not a useful model for the study of this virus infection.

(5) (With LBB) It has been known for some time that human adenoviruses will cause a choroiditis and ventriculitis in brains of monkeys inoculated intracerebrally with human adenoviruses. Attempts are being made to elucidate this phenomenon by use of certain specific fractions of the crude virus preparations prepared by cesium chloride density gradient centrifugation. It appears that purified virus particles will cause the pathologic changes. Further investigation is in progress.

Significance to Bio-Medical Research and the Program of the Division: These studies are designed to develop information which will allow control testing of new viral vaccines to be meaningful and assure safety of the product. The Additional Standards for Mumps Virus Vaccine and Rubella Virus Vaccine have been based on these studies.

Proposed Course of Project: It is expected that this program will expand during the next years as more new vaccines are developed.

Honors and Awards: None

Publications:

Pedreira, F. A., Tauraso, N. M., Palmer, A. E., Shelokov, A., and Kirschstein, R. L., Attempts to Infect Rhesus Monkeys with Human Type Adenovirus. Proc. Soc. Exp. Biol. and Med., 129:472-477, 1968.

Serial No. DBS 113
1. Pathology
2. Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: In vitro Study of Cells from the Central Nervous System

Previous Serial Number: 113

Principal Investigator: Ronald G. Clark, Ph.D.

Other Investigators: Joyce Johnson, LP-DBS

Cooperating Units: None

Man Years:
Total: 0.7
Professional: 0.2
Other: 0.5

Project Description:

Objectives: (1) To perfect methods of identification of cell types (astrocytes, oligodendrocytes and neurons) in tissue culture from the central nervous system. (2) To grow pure cultures of individual cell types from the central nervous system.

Methods Employed: Normal animals are sacrificed and specific areas of the brain aseptically removed for tissue culture. After removal, the tissue is treated in one of three ways: (1) untreated explants are grown in tissue culture, (2) lightly trypsinized tissue is grown in tissue culture, and (3) untreated tissue is ultrasonically vibrated and then grown in tissue culture.

After growth in tissue culture, the cell sheets (on coverslips) are removed and treated with special stains (gold and silver techniques). These stains, normally used on frozen sections of whole brain, will have to be modified in order to adequately characterize the cells.

Major Findings: Project is in its initial stage and has been slow to start while techniques are being developed.

Significance to Bio-Medical Research and the Program of the Division: The results of this study may enable the use of CNS tissue culture in studying the pathogenesis of neurotropic viruses.

Proposed Course of Project: This project has been discontinued because of prolonged leave of absence of the technician involved with this study.

Honors and Awards: None

Publications: None

Serial No. 131
1. Office of the Director
2. Contract Operations Office
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of Interferon Production

Previous Serial Number: 131

Principal Investigator: Stanley H. Singer, M.D.

Other Investigators: Ruth L. Kirschstein, M.D.
Michael Barile, Ph.D.

Cooperating Units: Laboratory of Pathology, DBS
Laboratory of Bacterial Products, DBS

Man Years:
Total: 2.0
Professional: 1.0
Other: 1.0

Project Description:

Objectives: The effect of chronic infections with certain viruses or mycoplasma on subsequent virus-cell interactions, with particular emphasis on interferon production, is under study.

Methods Employed: Cultures are first inoculated with certain viruses or mycoplasma in order to initiate chronic infection. The cultures are then superinfected with other viruses whose adsorption, penetration, replication, and ability to induce interferon is evaluated.

Major Findings: The principal findings indicate that mycoplasma infected cells have a decreased ability to produce and react to interferon, an antiviral protein naturally produced during the course of viral infections. Viral transcription in these cells was not affected. The net result of these changes was that mycoplasma infected cells were able to yield significantly higher amounts of virus than non-mycoplasma infected cells. The presence of mycoplasma also decreased the ability of cells to respond to a synthetic interferon inducer, namely, polyinosinic acid:polycytidilic acid.

Significance to Bio-Medical Research and the Program of the Division: The results indicate that the presence of a common cell contaminant, mycoplasma, can significantly alter virus-cell interactions. Because mycoplasma infected cells are capable of yielding greater amounts of virus than non-mycoplasma infected cells, it is possible that the course of naturally occurring viral infections in vivo may be altered by the presence of mycoplasma.

Proposed Course of Project: These studies will terminate at the end of this fiscal year when the principal investigator leaves to resume residency training.

Honors and Awards: None

Publications:

Singer, S. H., Kirschstein, R. L., and Baron, S., Viral Inhibition of Adenovirus 5 Treated Hamster Cells, Proc. Soc. Exp. Biol. and Med., 130:376-381, 1969.

Singer, Stanley H., Barile, Michael F., and Kirschstein, Ruth L., Enhanced Virus Yields and Decreased Interferon Production in Mycoplasma Infected Hamster Cells, Proc. Soc. Exp. Biol. and Med. (in press).

Singer, Stanley H., Kirschstein, Ruth L., and Barile, Michael F., Enhanced Vesicular Stomatitis Virus Yields from Mycoplasma Infected Hamster Cells, Nature (in press).

Serial No. DBS 133
1. Pathology
2. Animal Testing and Histopathology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of Marmosets (Saguinus mystax) and their Suitability for Biologics Testing

Previous Serial Number: 133

Principal Investigator: Douglas Lorenz, Ph.D., LP-DBS

Other Investigators: Ruth L. Kirschstein, M.D., LP-DBS
Marshall Ford, LP-DBS
Harold Baer, Ph.D., LBP-DBS
Lewellys Barker, M.D., LVI-DBS
David P. Stevens, M.D., LVI-DBS
John Petricciani, M.D., LP-DBS

Cooperating Units: Laboratory of Bacterial Products, DBS
Laboratory of Viral Immunology, DBS

Man Years:
Total: 3.8
Professional: 1.3
Other: 2.5

Project Description:

Objectives: The goals of this program are: (1) to maintain a colony of marmosets under laboratory conditions and (2) to describe the microbiology of that colony. From such work, a corps of personnel will become familiar with maintenance methods unique to these animals; also, means of recognizing and controlling marmoset diseases and microflora will become available. The pathogenesis of certain infectious diseases in marmosets will be elucidated.

Methods Employed: Sixty-one animals have been maintained in a 12 x 17 foot room where the mean temperature is 80°F. and the relative humidity is increased as much as possible with a portable humidifier. Colony maintenance is performed by a restricted number of persons to reduce contamination of the marmosets with agents from other primates housed nearby.

Classical methods of diagnostic microbiology have been employed to isolate and identify ectoparasites, helminths, protozoa, fungi, bacteria, Mycoplasma and viruses. Weights, rectal temperatures, microhematocrits, and differential white blood cell counts were obtained by classical methods.

Major Findings: (1) Colony maintenance: The colony of 86 Saguinus mystax was initiated September 27, 1967. In the first 10 weeks, 15 percent of the animals died from handling, shipping trauma, general debilitation assumed to be brought on by the stresses of captivity, and infestations with Prosthenorchis sp., a spiny-headed round worm which blocks and perforates the intestine. These worms are acquired when the animals eat cockroaches after captivity. Since that initial period, the rate of loss has averaged 3 percent in any 10-week period. Losses during the past year are attributed to filariasis, Prosthenorchis sp. infestation, hematomas, and experimentation. As of April 1, 1969, 53 marmosets (62 percent of the original colony) were available for investigations.

Maintenance procedures have been simplified so that personal requirements and animal handling are minimal. With reduced handling, heavier protective gloves, and more experience, the number of accidents requiring medical attention was markedly reduced.

Twelve pairs of animals are housed in special stainless steel cages (24" x 18" x 18") which were designed specifically for marmosets. Each cage is provided with a perch and a wooden nesting box. Pregnancies have occurred exclusively in this type of cage. The other cage is a standard rabbit cage. The animals live exclusively on 1/2 inch wire mesh. The larger cages are best for observing the animals.

Another critical requirement for Saguinus mystax maintenance is a relative humidity of 50 percent and a temperature of approximately 80°F. When humidity levels dropped to 20 percent for several days, the animals developed respiratory distress and weaker animals died. A virus has been isolated from throat swabs taken during this epidemic of coughing, sneezing, and malaise. The animals recovered quickly after the humidity was increased.

The final critical requirement for marmoset maintenance is strict vermin control. These insectivores can acquire parasite infections from cockroaches in the animal quarters.

(2) Nutrition: There are two basic diets: NIH monkey chow with 15 percent protein and Monkey Chow 25 (Ralston Purina Company, St. Louis, Missouri) with 25 percent protein, all of which is of vegetable origin. Both diets are supplemented with 1/4 of an orange per animal per week and 500 units of vitamin D_3 per day. The addition of multiple vitamins to the diet does not produce an obvious effect in the animals.

There was no obvious difference among the animals given the 15 percent or 25 percent protein diets until the animals were stressed by repeated bleedings and biopsy procedures. The average weight of the animals on 15 percent protein chow decreased about 9 percent (19.2 to 17.5 ounces) while the average

weight of the animals on 25 percent protein diet increased slightly during the same time and degree of stress.

We discovered an unexpectedly large requirement for water. Active animals consume about 1/4 of their body weight in water per day. Water consumption is a convenient indicator of animal vitality. The practice of putting vitamins or medication in the water most often results in restricted water intake so that the benefits must be weighed against the possibility of dehydrating the animals.

(3) Breeding: One set of triplets delivered in July, 1968 was stillborn. In January, 1969, two sets of twins and one single animal were delivered stillborn. Two other animals were certainly pregnant, but no fetuses were found. The animal found pregnant in July was again pregnant in January. We interpret this experience to indicate that there are two birth seasons per year which is comparable to the findings of others.

Based on experience with this single colony, we would advise the following for a given shipment of marmosets received in DBS: (1) at least 10 weeks should be allowed for the animals to adjust to their captivity before long-term experimentation begins; (2) a normal loss rate of 1 percent of the colony per month should be expected; and (3) births should not be expected before the animals have been maintained in routine fashion for more than one year and they would occur at 6-month intervals. From literature reports of fatal herpes virus infections acquired by marmosets from other South American primates, it is advised that new marmosets be placed in isolation for one month prior to contact with established colonies of marmosets in the event that the new animals have acquired a herpes infection during transit.

(4) Baseline studies: In August, 1968, the average weight of 62 marmosets was 20.1 ounces. Eight months previously the average weight of the same marmosets had been 18.8 ounces. In November and December, 1968, 59 readings from 29 animals gave an average rectal temperature of 39.6°C. with a standard deviation of ±0.4°C. The range was 38.4°C. to 40.5°C. The variation in a given animal is remarkably small. The mean microhematocrit value is 47 with a range of 25 to 59. These figures can be used with confidence as the colony has been stabilized.

(5) (With Doctors Barker and Stevens) Serum transaminase values are being determined as part of the pretest characterization of marmosets which will be used in a hepatitis study. The mean serum glutamic oxalacetic transaminase value is 97 with a standard deviation of ±26. The mean serum glutamic pyruvic transaminase is 31 with a standard deviation of ±9.6.

Sixty attempted biopsies with Menghini biopsy needles have realized 17 satisfactory liver specimens. All animals have survived the associated trauma, but there is some elevation in SGOT and SGPT values.

(6) (With Mr. Ford) During the initial stages of the colony, Prosthenorchis sp. was found in most of the animals. By January, 1969, no evidence of these intestinal parasites was found in any of the marmosets.

The incidence of animals with microfilaria in the blood has decreased from 83 percent in January, 1968 to 50 percent a year later. Various preparations of piperazine have not controlled these infections. The initial incidence of trypanosomes as determined from blood films was 42 percent. A year later no trypanosomes were seen in blood films, but by then culture methods had been developed and by this method the incidence was found to be 58 percent. The trypanosome was found not to be Trypanosoma cruzi. Mice, rats, guinea pigs, and rabbits were found to be insusceptible to this marmoset parasite.

Bacteria similar to Salmonella or Shigella have disappeared from the colony. Proteus sp. are found in all stool specimens now and from most oral swabs. No Mycoplasma were isolated. No viruses have been isolated from routine stool specimens, oral swabs, or biopsy specimens using rhesus monkey kidney, vervet monkey kidney, HEP-II, and rabbit kidney cell cultures. During one epidemic of respiratory distress when the relative humidity in the room dropped to less than 20 percent, a virus-like agent was isolated from oral swabs from ten animals. This agent is being identified.

(7) (With Dr. Baer) In a study concerning delayed hypersensitivity, fluorodinitrobenzene (DNFB) in complete Freund's adjuvant containing heat killed BCG organisms was inoculated subcutaneously into three marmosets. Three other animals were inoculated with material which was identical to that above except that dinitrophenyl bovine serum albumin was substituted for DNFB. The animals were tested intradermally with purified protein derivative (PPD) and cutaneously with DNFB. It was found that two of three animals developed delayed hypersensitivity to DNFB, while five of six animals developed delayed hypersensitivity to PPD. The tests demonstrated that the Saguinus mystax will react to PPD so that tuberculin screening is probably an effective means of monitoring the colony for tuberculosis.

(8) (With Dr. Petricciani) Marmoset kidney cell cultures can be grown for many passages without showing cytopathic effects. Cells from fetal thymus and lung were also grown.

The lack of cytopathic effects in cultures indicates that the animals are relatively free of viruses.

One kidney was removed surgically from a male marmoset, and cell cultures were established by classical procedures and have progressed through 13 passages in four months. In exploratory experiments the cells have been passed 20 times. The growth rate, plating efficiency, karyology, virus susceptibility, antigenic characteristics, and oncogenicity of these fibroblast cells are being studied. The donor animal has survived and is available for homotransplantation studies.

Significance to Bio-Medical Research and the Program of the Division: This work will contribute to any evaluation of the marmoset as a subject for biologics testing. The small size of this primate would allow much more economical biologics testing. Cell cultures derived from this primate allow

the possibility of establishing new markers for viruses used in human vaccine production. Diploid cell cultures derived from these animals can be used to demonstrate the feasibility of producing diploid animal cell lines.

Proposed Course of the Project: The major effort will be directed toward maintaining the health of the colony while conducting experiments with infectious agents. The immediate experiments will involve a description of the pathology of hepatitis in the marmoset.

We will continue to investigate means of increasing the birth rate and survival of newborns. We will continue to investigate marmoset cells in culture as means of characterizing viruses.

Honors and Awards: None

Publications: None

Serial No. DBS 161
1. Pathology
2. Animal Testing
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Chromosomal Effects of in utero Viral Infections on Sub-human Primates

Previous Serial Number: None

Principal Investigator: John C. Petricciani, M.D.

Other Investigators: Ruth L. Kirschstein, M.D.

Cooperating Units: None

Man Years:
Total: 0.4
Professional: 0.25
Other: 0.15

Project Description:

Objectives: The relationship of early intrauterine infection by certain viruses to various congenital abnormalities is well documented. The possible role of other viruses in the development of an abnormal karyotype in the fetus of a sub-human system is being investigated.

Methods Employed: Cells from stump-tailed monkeys (Macaca speciosa) are established in tissue culture and screened for susceptibility to a variety of non-oncogenic viruses. Appropriate selections are then made to infect a pregnant monkey and monitor the karyotype of the fetus.

Major Findings: None to date

Significance to Bio-Medical Research and the Program of the Division: These studies may provide evidence for the viral etiology of an abnormal karyotype in a sub-human primate system with possible significance for man.

Proposed Course of Project: These studies will continue in the coming year.

Honors and Awards: None

Publications: None

Serial No. DBS 162
1. Pathology
2. Animal Testing
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: The Effect of Bacterial Viruses on Animal Virus Infectivity and on Animal Cells

Previous Serial Number: None

Principal Investigator: John C. Petricciani, M.D.

Other Investigators: Carl R. Merril, M.D.
Gerald Rosen, M.D.

Cooperating Units: Laboratory of General and Comparative Biochemistry, NIMH

Man Years:
Total: 0.25
Professional: 0.25
Other: 0.0

Project Description:

Objectives: The potential role of selected bacterial viruses in inhibiting animal virus replication in vitro is being investigated. In addition, the effects of bacterial viruses themselves on animal cells is being studied.

Methods Employed: Individual members of a group of well-characterized bacterial viruses are being fractionated and the components tested for anti-viral activity in animal cells. Intact bacterial viruses are inoculated onto animal cell culture systems which are then observed for changes and assayed for bacterial virus periodically.

Major Findings: None to date

Significance to Bio-Medical Research and the Program of the Division: These studies may provide information on the possible use of components of bacterial viruses as anti-animal virus agents and data on their biologic potential in animal cells is being assessed.

Proposed Course of Project: These studies will continue in the coming year.

Honors and Awards: None

Publications: None

Annual Report of
Laboratory of Viral Immunology
Division of Biologics Standards
July 1, 1968 through June 30, 1969

Laboratory Chief's Summary

The program of the Laboratory of Viral Immunology can be described under three headings: (1) research, (2) control testing and (3) advisory support. Research represents the major responsibility of the Laboratory in terms of man-hours of effort. About two-thirds of the work could be described as research or research-associated. Control testing involves the assay of licensed or experimental biologics products provided by commercial concerns and the standardization of reference materials distributed for use in biologics control. The staff of the Laboratory supports the Office of the Director in an advisory capacity in such matters as licensing of products, research contracts operations, investigational new drug applications and investigation of reports of possible adverse effects related to the use of biologics.

The research program is far-ranging; the only common denominator is the relation of the information sought to current or potential needs in the biologics field. Activities in this area will be highlighted in the following paragraphs:

Rubella. This program begun by the Laboratory's staff in 1963 should in the coming months provide a maximum return on invested effort. Two research achievements by our group, the attenuation of the virus in 1965 and the development of the HI test for immunity in 1966, served as a catalyst to final vaccine development. Rubella vaccine is expected to become commercially available before the end of fiscal 1969. The current rubella research of the LVI is described in some detail in the individual project reports. Particularly important have been: laboratory and clinical characterization of a number of attenuated strains including each of the vaccines being considered for license; vaccine studies involving women (rubella-like reactions to the attenuated strains occur uniquely in adult females); and development of a laboratory model for the study of vaccine-induced immunity and resistance to reinfection.

In the area of control testing a rubella reference serum and a reference virus have been prepared. The serum has been standardized and distributed. The virus preparation is currently under test. Techniques being used by the potential manufacturers for production, potency assay and safety testing are being evaluated. In this way the reliability of their procedures will be ascertained well in advance of license.

We expect that the smooth transition from the experimental phase to license and general clinical use of vaccine will at least in part be due to our program begun six years ago in anticipation of future need.

Hepatitis. The control of hepatitis remains as one of the major challenges in virology. Its importance in the biologics field is two-fold: (1) some forms of hepatitis are transmitted by biologics products (blood and blood derivatives) and (2) if a vaccine were developed it might be possible to prevent hepatitis.

The causative virus or viruses of hepatitis have long eluded scientists. Over the years one or another false leads have produced brief optimism followed by disappointment. Because of the importance of the problem the LVI has maintained a small program of exploratory research. During the past year promising preliminary results have come from this effort. It appears that a virus-like material suspected of being the etiologic agent can be demonstrated by an immunologic technique and by electromicroscopic examination. If these particles are hepatitis virus then the availability of assay methods should speed research. For example, the methods would be of considerable assistance in the search for a method of culturing the virus.

Smallpox Vaccine. Responsible groups are increasingly concerned about the background of morbidity and mortality associated with the use of smallpox vaccine in relation to the decreasing risk of importation of the disease. Our pro gram has anticipated this trend and for several years been committed to the study of variants of vaccinia virus and their possible value for immunization. Progress in this work is described in the individual project reports.

Measles and Mumps Vaccine. Major research efforts of the past were devoted to the study of these two vaccines as experimental products. Now in routine commercial use and recommended for administration to every American child these vaccines present continuing control obligations to the Division. During the past year perfection and application of a sensitive microtiter assay system has remarkably improved and simplified our potency testing of the products. This and similar test development projects are an important part of the Laboratory's research program.

Herpes-like Viruses. A great deal of attention has centered around the herpes-like virus, generally termed Epstein-Barr (EB) virus discovered several years ago in cultured cells derived from Burkitt lymphoma. The Section on Viral Genetics of the LVI has concentrated all of its effort on the study of the virus and its possible role in infectious mononucleosis and certain malignancies. Of immediate interest to the Division has been the principal investigator's demonstration of the association of EB virus presumably contained in transfused blood with the post-perfusion syndrome, an infectious nononucleosis type of illness.

Hereditable Diseases. As the study methods of modern medicine have become more sophisticated, a striking array of hereditable diseases have been recognized. Generally the traits involved are recessive and mothers may given birth to normal children, diseased children (homozygous) or carriers of the trait (heterozygous). The Cell Metabolism Unit of the Laboratory is exclusively concerned with the application of cell culture techniques to genetic studies. This work has been in part of a fundamental nature seeking to chart the nature of the biochemical defect determined by the genetic flaw. However, a particularly excited outgrowth of the basic research has been use of the techniques to establish an intra gestational diagnosis. Here amniotic cells aspirated in early pregnancy are cultured and studied to determine if the unborn infant will or will not have the disease. These results when positive can be used as a basis for the medical interruption of pregnancy.

Cytomegalovirus. These highly species specific viruses are found in many animals as well as man. Simian cytomegaloviruses are common contaminants of monkey kidney cell cultures. Because of the use of monkey kidney cultures for vaccine manufacture it is important to acquire information that could be used to detect potentially contaminated animals. The Laboratory has found that the epidemiology of simian cytomegalovirus resembles that of SV_{40}, another common virus of monkeys encountered in early work with poliovirus vaccines. Few monkeys harbor the virus in the jungle thus the rapid transport and quarantine methods developed to import monkeys free of SV_{40} and measles should furnish animals free of cytomegaloviruses.

LABORATORY OF VIRAL IMMUNOLOGY

Control Program

July 1, 1968 through June 30, 1969

Antibody Assays: Using virus neutralization or hemagglutination-inhibition tests.

Immune Serum Globulin (Human)

Number of lots received	114
Poliovirus potency tests	73
Number of tissue cultures	15,330
Measles potency tests (HAI)	71
Number of titrations	142

Poliomyelitis and Rh (D) Immune Globulin (Human)

Number of lots received	13
Potency tests	13
Number of tissue cultures	2730

Testing of Reference Materials:

NIH Measles Reference Serum No. 1

Number of tests (HAI)	9
Number of titrations	18

NIH Reference Poliomyelitis Immune Globulin Lot 174

Number of tests for polio antibodies	14
Number of tissue cultures	2940

NIH Reference Poliomyelitis Immune Globulin Lot 175

Measles HAI tests	9
Number of titrations	18

Poliomyelitis Reference Serum IIA-4

Number of tests	15
Number of tissue cultures	3750

Vaccinia Immune Globulin

Number of tests	11
Number of tissue cultures	2750

Testing of Vaccines, Live, Attenuated:

Poliovirus, Oral (Screening of pre-inoculation monkey sera)

Number of neurovirulence tests	22
Number of sera	764
Number of tissue cultures	5348

Testing of Vaccines, Inactivated:

Diphtheria, Tetanus, Pertussis and Poliomyelitis

Poliomyelitis

Number of lots received	1
Number of monkey safety tests	6
Pre-inoculation sera	60
Post-inoculation sera	96
Number of tissue cultures	1092
Monkey potency tests on polio component	2
Pre-inoculation sera	24
Post-inoculation sera	24
Number of tissue cultures	3724

Potency and Marker Tests (Live, Attenuated Vaccines):

Poliovirus, Oral

Number of lots received	
Monovalent lots tested for virus content and genetic markers	13
Number of tissue cultures	2600

Measles

Vaccine lots tested	23
Number tube titrations	32
Number microtiter plate titrations	18

DBS Reference

Number tube titrations	42
Number microtiter plate titrations	16

Mumps

Vaccine lots tested	22
Number tube titrations	34
Number microtiter plate titrations	16

DBS Reference

Number tube titrations	70
Number microtiter plate titrations	9

Rubella

Vaccine lots tested	13
Number tube titrations	9
Number microtiter plate titrations	4

LABORATORY OF VIRAL IMMUNOLOGY

Production of Cell Cultures for Use in Control and Research Activities

July 1, 1968 through June 30, 1969

	Tubes	2 oz.	8 oz.	32 oz.	Microtiter Plates	Suspension Cultures
BS-C-1	6220	25	37	45		
Vero	505	440	14	37	52	
RK-13	1620	27	--	4	2	
BHK-21		237	19	17		
Primary Chick		1705		203		
Primary Duck		637				
Guinea Pig Kidney	180	110	4			
Human Foreskin	100	138				
Bone Marrow	320					
Rhesus Kidney (Stable FE-8)	50	104				
NHDL-3						48 x 20 ml
Pig Synovium	100	250				
Human Synovium	215					

Serial No. DBS 49
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigations concerning rubella virus and vaccine.

Previous Serial Number: DBS 49

Principal Investigator: Paul D. Parkman, M.D.

Other Investigators: Hope E. Hopps, M.S.
Thomas E. Hobbins, M.D.
H. Elliott Larson, M.D.
William J. Davis, M.D.
James P. Simsarian, M.D.
Barbara C. Meyer, M.S.
Joan C. Enterline, A.B.
Robert D. Douglas
Eva R. Brown, B.S.
Harry M. Meyer, Jr., M.D.

Cooperating Units: Walter Reed Army Medical Center, Department of Obstetrics and Gynecology
Walter Reed Army Institute of Research, Department of Virus Diseases

Man Years:

Total:	11.4
Professional:	7.0
Other:	4.4

Project Description: Part I. Studies of the biological characteristics of rubella virus.

For the past several years the major efforts of our laboratory have been concerned with the development of attenuated rubella virus strains, the production of experimental vaccines and the clinical testing of these preparations in man. During the course of those studies it became essential to develop methods for identification and quantitative measurements of virus as well as to develop tests which would serve as indicators of virus modification.

Now that many groups have successfully tested vaccines prepared in a variety of avian and mammalian cells, our attention has turned to procedures which can be used in the monitoring of licensed live rubella vaccines, In general, many of the procedures established with live polio, measles and mumps vaccines can be employed. However, specific tests must be used for rubella vaccines with respect to virus identity, quantitation of antibodies, vaccine

potency measurement and markers of attenuation.

Just as in the development of live attenuated polio and measles virus vaccines, the absolute need for laboratory marker tests in developing an attenuated rubella virus vaccine was recognized at the outset. The several tests that were developed will now be useful in the course of vaccine production, particularly for potency assay and for testing of seed virus.

Assay for Potency of Vaccines. Potency testing of the HPV series of experimental vaccines prepared in our laboratory in primary African green monkey kidney cells (GMK) was carried out by infectivity titration in GMK cells and in RK_{13} continuous rabbit kidney cells. A series of potency tests of experimental rubella vaccines grown in other types of cell culture, including those prepared in chick embryo, duck embryo, dog and rabbit kidney cells, were performed in both the GMK and RK_{13} cell systems. Each of the vaccines, regardless of the cell substrate to which it had been adapted, multiplied to comparable titers in GMK and RK_{13} cells. The RK_{13} cell system was somewhat more sensitive to virus than was the GMK; geometric mean titers were usually 0.5 $\log_{10}$ higher in the rabbit kidney cells.

In studies performed earlier, dilutions of HPV-77 were administered to children by the subcutaneous route. Aliquots of the same vaccine were also titrated in GMK and RK_{13} cells. The results indicated that one to ten tissue culture infectious doses of virus represent one human infectious dose when the vaccine is given subcutaneously. Thus, the use of GMK and RK_{13} cells provides an accurate potency assay system and one which can be correlated with immunogenicity for man.

Marker Tests. The marker procedures originally developed in our laboratory for detecting modification in the HPV vaccines have also been applied to the testing of vaccines prepared in duck embryo, canine kidney, rabbit kidney and chick embryo cell culture propagated viruses. These vaccines all behaved similarly to HPV-77; rapid and complete cytopathic effects (CPE) were produced in monolayer cultures of RK_{13} cells, and plaques formed in agar-overlayed RK_{13} cultures. Virulent viruses, in contrast, failed to produce complete CPE or plaques in RK_{13} cells.

Interferon production served as an additional in vitro marker test. Induction of interferon by virulent rubella virus was reported by Neva and Weller in 1964. Since increased interferon production was associated with attenuation of several viruses, including polio and measles viruses, the M33 strain at several levels, from the second to the 166th passage, was examined for its capacity to induce interferon in GMK cells. A second strain, designated ML, was also examined at second and 20th passage levels. Interferon titer increased with virus passage. Between second and 20th passages only low levels were detected, but by passage 66, 2 to 8-fold increases were noted. Increased titers after the 100th passage were not seen and by 166th passage there was a consistent 8-fold increase in titer over that of the parent strain. The ML strain behaved similarly showing a 4-fold increase in titer after 65 passages.

Interferon induction by derivatives of HPV-77 in duck embryo, canine

kidney and chick embryo as well as the Cendehill strain prepared in rabbit kidney were studied not only in GMK, but also in the cell system to which the virus had been adapted. Little interferon was detected in chick, duck or dog tissue under our conditions of production and assay, but each of these HPV-77 derivatives produced high interferon levels in GMK. The Cendehill strain, in contrast, failed to induce interferon in GMK, but high titers were obtained when primary rabbit kidney cell cultures were used. Thus, it has been possible to adapt each of the in vitro marker test methods to the differentiation of high passage level attenuated vaccine strains from low passage virulent virus.

Studies on Rubella Virus Adaptation to Chick Embryo Cell Cultures. Live virus vaccines for yellow fever, measles and mumps prepared in embryonated eggs or chick embryo cell cultures have been safely administered to many millions of persons. For this reason, we and others have attempted to prepare rubella vaccines in chick embryo cells. In this work we have encountered two problems: (1) difficulties in adapting rubella virus to serial propagation in chick embryo cell cultures and (2) excessive virus modification (overattenuation) after relatively few chick embryo passages.

One year ago we reported the successful adaptation of rubella virus to chick embryo cell cultures and the clinical trial of an experimental vaccine at the 10th cell culture passage. This material proved to be overattenuated. Since animal experiments had shown lower chick embryo passages to be immunogenic, methods were developed for producing adaptation after one passage.

A wide range of green monkey kidney cell culture passage levels of rubella virus have been inoculated into chick embryo cells. Serial propagation was not achieved in 2 attempts with virulent 5th passage virus nor in 8 attempts with attenuated viruses at the 68th to 78th passage levels. At the 120th GMK level one of four attempts was successful. Using the 150th, 166th and 185th GMK passages, adaptation has been uniformly successful. Thus, the capacity of rubella virus to adapt to chick embryo cultures is related to the number of passages it has undergone in GMK.

Even in the first chick embryo passage, rubella virus proliferation and persistence were related to the green monkey kidney passage level of the inoculum. Use of 29°C incubation temperatures has permitted prolonged maintenance of the chick embryo monolayers for as long as 20 weeks. Sixty-eight GMK passage virus proliferated only to a limited extent and by four weeks the titer had fallen to undetectable levels. One hundred twentieth GMK passage virus persisted in diminishing titers for up to 15 weeks. In contrast, both 166th and 185th GMK passage virus proliferated well; maximum titers of $10^{4.3}$ and $10^{4.9}$ were attained between the 4th and 6th weeks after inoculation. The HPV-166 passage level virus was selected for further studies.

During the course of a first passage in chick embryo cells, there was a gradual shift in the properties of the virus. Cultures inoculated with HPV-166 virus were incubated for prolonged periods at 29°C. After 2, 8, 10 and 18 weeks virus was harvested from this first chick embryo passage and subcultured on new chick monolayers. Only about 10 TCD_{50} of virus was present in fluids harvested from the 1st passage cultures after 2 weeks and no multiplication occurred in the 2nd chick embryo culture passage. Virus removed at 8 weeks

titered $10^{1.5}$ and limited propagation took place in the 2nd passage. In contrast, virus harvested after 10 or more weeks, while no higher in titer after its first passage in chick embryo cells, grew to high titer on 2nd passage and could be propagated with ease thereafter.

HPV-166 rubella virus was propagated through 5 serial passages in chick embryo cells. The 10 weeks virus harvest from the 1st passage was well adapted to chick embryo; satisfactory growth occurred in each subsequent passage. However, maximum virus titers of $10^{3.1}$ to $10^{3.7}$ were achieved only after the 2nd passage. These results indicated that production of a vaccine at the fifth passage level was technically feasible.

Testing of Duck Embryo Cell Cultures for Extraneous Agents. The development of rubella virus vaccines attenuated and produced in duck embryo cell cultures (DETC) by Buynak et al (Buynak, E.B., Hilleman, M.R., Weibel, R.E. and Stokes, J., Jr.: Live Attenuated Rubella Virus Vaccines Prepared in Duck Embryo Cell Culture. I. Development and Clinical Testing. J.A.M.A. 204: 103, 1968) created an interest in the safety of this particular cell system as a substrate for the preparation of live attenuated virus vaccines, and specifically in its freedom from detectable extraneous microbial agents. Eleven lots of duck embryo cells were prepared, employing the same source of cells used by the vaccine manufacturers. Cultures were prepared from 7 to 13 day old embryos, and were serially subcultured as cell lines at 7 day intervals. At each passage level cultures were examined for evidence of cytopathic effect (CPE), hemadsorption with chick and guinea pig erythrocytes, interference with vesicular stomatitis and Sindbis viruses and for development of intranuclear or intracytoplasmic inclusions in fixed Giemsa-stained preparations. Three lots of these cells were used for the production of rubella virus seed pools and experimental vaccine production. Culture fluids from these lots were subpassaged in DETC, green monkey kidney, BS-C-1, RK_{13} and human embryonic kidney cell lines for evidence of extraneous cytopathic, hemadsorbing or interfering agents. Results of these studies were entirely negative. No agents were detected by any of the various procedures used. Thus, our experience to date indicates that DETC appear to provide a useful system for vaccine production.

Influence of Cell Type and Incubation Conditions on Rubella Virus Hemagglutination (HA) Production. Reports that high titers of rubella virus could be obtained from the continuous African green monkey kidney Vero cell line led us to examine the utility of these cells for rubella HA production. Cultures of BHK-21 and Vero cells were grown either in 1 liter bottles incubated on a roller apparatus, or in 8 oz. milk dilution bottles. Viruses adapted by seven serial passages in BHK-21 cells or 13 passages in Vero cells were used to inoculate these two cell types, in order to examine the effect of virus adaptation to one or the other cell line on HA production. Roller cultures received a 10 ml inoculum and stationary cultures received three ml. After adsorption for two hours the inocula were removed, fresh Eagles minimum essential medium added and the cultures incubated at 35°C. Medium was changed daily and supernatant fluid harvests were assayed for HA activity. No striking differences were observed which were consistently related to the cell culture adaptation of the inoculum; HA yields of 1:8 or greater were obtained in cultures receiving either Vero or BHK-21 adapted virus. Incubation in the roller drum enhanced HA titers in BHK-21 cell cultures, but had no effect on yields from

Vero cells. It seems likely that this phenomenon was related to the cell growth promoting effects of roller incubation on BHK-21 cells, resulting in a higher cell density and a greater antigen production. The data indicated, however, that 4-fold higher HA titers were produced in the BHK-21 cell cultures. Maximum yields from BHK-21 cultures ranged from 1:64 to 1:128, while the highest HA titers of harvests from Vero cultures were from 1:16 to 1:32. This finding was confirmed in other experiments; in which BHK-21 cell HA levels were 8-fold higher. These findings indicated that the use of roller cultures of BHK-21 cells provided a reliable system for producing rubella HA antigens of high titer, and failed to show any advantage in the use of Vero cells.

Identification of Rubella Viruses by Hemagglutination-inhibition (HAI). Earlier techniques for the specific identification of rubella viruses depended on cell culture assays of the neutralization of virus by antisera containing rubella antibodies. This procedure was time consuming and technically demanding. With freshly recovered virus strains in early cell culture passage it is frequently difficult to predict infectivity titer, and retesting is frequently required before the 10-50 $TCID_{50}$ test dose range necessary for this type of test is attained. With the development of the rubella HA test in our laboratory, it has been possible to devise a simpler procedure. Here the virus to be tested is subcultured into BHK-21 cells grown in 2 oz. plastic bottles. Cultures are incubated at 35°C; the medium is changed after 24 hours incubation and at 3-4 day intervals thereafter. At 4 and 8 days after inoculation supernatant culture fluids are tested for HA activity. Ordinarily, rubella virus will produce detectable HA activity in the first passage in BHK-21; cells; HA titers are usually higher in fluids harvested at 8 days than in those tested earlier. Thus, in a representative experiment involving interfering virus isolates recovered from vaccinees, production of HA antigens was achieved in the first BHK-21 cell passage in all of 6. HA levels ranged from 1:4 to 1:16 at 8 days after inoculation. Occasionally isolates will require an additional BHK-21 cell culture passage before HA is detected. The HA antigens thus produced were then identified by HAI with specific rubella immune sera prepared in rabbits. Four HA units of antigen were tested against heat-inactivated, kaolin and chick red blood cell (CRBC) adsorbed antibody positive and negative rabbit serum. Included in each test were repeat titrations of the HA antigens and serum - CRBC and diluent CRBC controls. HA inhibited by the known rubella antiserum and unaffected by the antibody negative serum was thus shown to be rubella virus. This new method has provided a less complex, less expensive and more rapid means for identifying rubella viruses.

Project Description: Part II. Studies of rubella virus infection in man.

During the past year several experimental rubella virus vaccines produced in our laboratory and elsewhere have been evaluated for immunogenicity and clinical acceptability in children and in adult women. Studies involving children and young adolescents were performed at the Arkansas Children's Colony, Conway, Arkansas, and the projects involving adults was carried out in cooperation with the Walter Reed Army Medical Center's Department of Obstetrics and Gynecology.

Clinical Trial of Cendehill Strain Experimental Rubella Virus Vaccine. The Cendehill vaccine, supplied through the courtesy of Smith, Kline &

French Laboratories, was prepared in primary rabbit kidney (RK) cell cultures. This material had received 4 passages in primary African green monkey kidney (GMK) cells followed by serial subcultures in RK_{13} cultures. The titer of the vaccine was $10^{3.8}$ per 0.5 ml human dose. Twenty children at the Arkansas Children's Colony participated in the study. Each subject was examined for evidence of clinical reactions, and throat swabs were collected daily for the 28 days following vaccination. Blood specimens were obtained at 1 to 2 week intervals for serologic study. No clinical reactions suggestive of rubella were noted following vaccination; none of the 20 children developed fever, rash, lymphadenopathy, respiratory or arthritic symptoms. Minor local reactions were noted in 5 of the 20 vaccinees. These reactions consisted of transient induration and redness at the inoculation site; all such local lesions disappeared within 2 to 3 days after inoculation without further sequelae and none appeared to be associated with allergic manifestations. Sixteen of the 20 vaccinees (80%) shed virus in the pharynx during the period from 8 to 26 days after immunization. The average duration of virus shedding among these 16 subjects was 3.5 days. All of the inoculated children developed HAI antibody by 28 days after immunization. Geometric mean antibody levels in the group was 1:66; these levels were only slightly lower than those observed with the HPV-77 and HPV-77 derivative vaccines prepared in duck embryo or dog kidney cell cultures (1:100-1:160). None of the 9 susceptible children living in close contact with the vaccinees developed clinical or virologic evidence of infection. Thus, in children the Cendehill vaccine appears to be attenuated and noncommunicable to contacts. This virus strain appears to be slightly less immunogenic than the HPV-77 derived vaccines; whether this is of importance will only be determined by prolonged followup of vaccine recipients for persistence of antibody and protection against the natural disease.

Rubella Virus Vaccination of Adult Women. Although the primary use of rubella virus vaccines, when available, will be in children and adolescents, there will be an interest in protecting the approximately 10% of adult women who are at present rubella-susceptible. The use of attenuated live rubella virus vaccines in women, however, was reported by Krugman to be associated with the development of clinical reactions, including rash, lymphadenopathy and the occurrence of arthralgia and arthritis (Cooper, L.Z., Ziring, P.R., Weiss, H.J., Matters, B.A. and Krugman, S.: Transient Arthritis after Rubella Vaccination. Am. J. Dis. Child. July-Aug. 1969, in press).

There was felt to be a need to obtain more data relating to the use of rubella virus vaccines in women and a study was designed to evaluate the influence of several factors on these reactions including: (1) the degree of virus attenuation, (2) the effect of simultaneous vaccination and administration of gamma globulin and (3) the safety and effectiveness of rubella vaccines at several levels of virus attenuation.

Women were considered for participation in this study if they were devoid of antibodies to rubella virus, were normal, healthy women living in Washington, D.C. area eligible for care at armed forces hospitals, were not pregnant, and were using adequate methods of birth control for duration of study.

Four small groups of women received (1) the HPV-77 vaccine passaged five times in duck embryo cell cultures (HPV-77 DE5); (2) the same vaccine given

simultaneously with gamma globulin 0.02 ml/lb body weight; (3) the Cendehill strain prepared in primary rabbit kidney cells (Cendehill GMK4 RK53) and (4) an HPV-77 derivative prepared in chick embryo cell cultures (HPV-150 CE10). This material had been shown earlier to be "overattenuated" in children.

A daily temperature record was kept and physicians made home visits three times weekly through 4 weeks after vaccination. Throat swabs, pertinent histories and physical examination were performed on these visits. Blood specimens were obtained prior to vaccination and at 4 and 8 weeks after vaccination for antibody tests.

Virus isolations from throat swab specimens demonstrated that 4 of 5 women receiving HPV-77 DE5 with or without globulin had detectable virus in similar specimens. Active immunity as measured by development of HAI antibodies was achieved in all women receiving the Cendehill vaccine, in 2 of 3 receiving HPV-77 DE5 and in all of 4 given HPV-77 DE5 with gamma globulin. None of the women receiving the highly attenuated HPV-150 CE10 vaccine shed virus or developed antibody.

None of the women in the study experienced fever. One subject developed a rash; this woman received HPV-77 DE5 and gamma globulin. Lymphadenopathy was observed in 3 of 5 receiving the Cendehill strain. One of 5 of these patients experienced mild arthralgias; there was no arthritis in this group. Lymphadenopathy was observed in 6 of 8 women receiving HPV-77 DE5 or HPV-77 DE5 and gamma globulin. Arthralgia were reported in 5 of 8 women and 3 of the 8 had signs of arthritis: 2 in the proximal interphalangeal and metacarpal phalanged joints of the hands and one in the left knee. The arthritis was mild, self-limited and of short duration in all. There were no reactions observed among those women given the nonimmunogenic HPV-150 CE10 preparation. It is interesting to note that 2 of 3 women who received gamma globulin in attempt to provide passive protection developed arthritis. Thus, this indicated that globulin administration was ineffective in modifying vaccine reactions.

This experience supports the data of others confirming the immunogenicity of the HPV-77 DE5 and Cendehill viruses. Although the numbers of vaccinees are small, the data provides suggestive evidence that the Cendehill strain is less likely to cause vaccine reactions in adult women that HPV-77 DE5 vaccine. It is important to note, however, that with either preparation, reactions were mild, self-limited and well tolerated by the vaccinee.

Production and Clinical Trial of the HPV-166 Chick Embryo 5 Experimental Rubella Virus Vaccine. Three vaccine lots, four to six liters in volume, were prepared with titers adequate to insure final container potency in excess of 1,000 $TCID_{50}$/0.5 ml dose. Prolonged incubation at low temperature allowed multiple harvests from a single cell lot. Daily harvests could be made without affecting titers. Safety testing of bulk, control and final container fluids was carried out in accord with regulations for licensed rubeola vaccine produced in chick embryo cell cultures.

Seventeen children at the Arkansas Children's Colony were subcutaneously inoculated with 0.5 ml of the HPV-166 CE5 experimental rubella virus vaccine. None of the children suffered clinical reactions that could be attributed to

the vaccine. Antibodies appeared in 59% of the vaccinees; the geometric mean titer was 1:37. Nasopharyngeal virus shedding occurred between the 13th and 28th days in 7 of the 10 subjects who developed antibodies. The appearance of antibodies was delayed; hemagglutination-inhibiting antibody developed between the 28th and 56th days after inoculation.

The rate of seroconversion obtained with this vaccine was lower than that obtained with the previously tested chick embryo produced experimental rubella virus vaccine (HPV-150 CE10) although average antibody titers appeared to be slightly higher. Neither chick embryo vaccine produced the high seroconversion rage achieved by HPV-77, our original attenuated vaccine produced in green monkey kidney cells.

Project Description: Part III. Studies directed toward the standardization of serologic tests for rubella.

The Rubella Reference Serum, lot 1, prepared from a pool of serum obtained from adult donors has been titrated repeatedly in neutralization and hemagglutination-inhibition tests. The geometric mean antibody titer in a series of eight determinations of neutralizing antibody was 1:53. More extensive testing has been performed using the hemagglutination-inhibiting (HAI) antibody test, since this assay technique has replaced the neutralization method as the mainstay in rubella serologic testing. A total of 91 tests have been performed using the heat inactivated, kaolin-chick red blood cell adsorbed reference serum. The distribution of the individual values was as follows: 1:32, 2; 1:64, 38; 1:128, 43; 1:256, 7; and 1:512, 1. The geometric mean HAI titer was 1:100. This reference serum is being distributed to laboratories for use in standardizing rubella antibody test procedures.

Project Description: Part IV. Studies of the antigenicity of inactivated preparations of rubella virus.

The production of virus preparations of high infectivity titer and the development of methods for detecting hemagglutinins (HA) provided new means for studying the inactivation of rubella virus. Formalin was added in a final concentration of 1:4000 to virus which had been produced in BHK-21 cell cultures. The formalin-containing preparation was then filtered through millipore membranes of 450 mμ average pore diameter. Samples were taken before the addition of formalin, immediately after the filtration procedure and after formalinization for 4, 6, 8, 10 and 30 hours incubation at 37°C. The formalin was neutralized by the addition of sodium bisulfite, and samples were titrated for residual virus and hemagglutinin. A total of 8 such preparations have been studied. Infectious virus was rapidly inactivated; detectable residual infectivity was commonly present in specimens collected at 6 hours, but not in those taken after 10 hours. Based on these observations, materials were incubated for up to 30 hours, approximately three times the period demonstrated to be necessary to reduce the concentration of live virus to the point where no virus was detectable in the GMK titration system. However, when approximately 400 cc of each lot was tested in GMK and RK_{13} cells after formalinization for 30 hours, each lot contained small amounts of demonstrable residual virus. As might be expected, HA was much less easily inactivated; two- to four-fold reductions in HA titer were observed during the 30 hours of incubation with formalin. Ob-

viously more prolonged incubation periods will be required to produce fully inactivated materials. The HA levels may provide a useful index of the immunogenicity of inactivated preparations.

Project Description: Part V. Studies of possible rubella virus oncogenic effects.

No work on oncogenicity studies was performed during this reporting period.

Project Description: Part VI. Studies of rubella virus infections in experimental animals.

Studies of Rubella Virus Immunogenicity in Rhesus Monkeys. The antigenicity of chick embryo cell culture (CETC) adapted rubella virus preparations was evaluated in rhesus monkeys; results were assessed in terms of seroconversion rates and antibody titers. The HPV-150 series was serially passaged at 2 week intervals in cultures incubated at 32°C. Ninety-three percent of monkeys inoculated with the 7th chick embryo passage of this material developed antibodies, while only 50% of the animals inoculated with the 10th passage responded. This reduction in seroconversion rate was accompanied by an 8-fold reduction in geometric mean antibody titer.

The HPV-166 series was passaged at 6 to 10 week intervals in CETC incubated at 29°C. Monkeys receiving third passage virus all developed high levels of antibody; the geometric mean was 1:355. Monkeys receiving chick embryo 5 material had a 90% seroconversion rate. However, the antibody levels were quite low. In both passage series, the lower CETC levels were immunogenic; 2 or 3 additional passages, however, effected a significant reduction in immunogenicity for monkeys. Thus tests of these preparations in rhesus monkeys has shown that less of immunogenicity occurred rapidly with serial passage.

Evaluation of Vaccine Induced Immunity: Rubella Virus Challenge Experiments in Rhesus Monkeys. Rhesus monkeys were used to study the mechanism of anamnestic antibody responses in vaccinated animals after natural rubella virus challenge. Monkeys infected four to six months earlier with HPV-77, other attenuated rubella strains, or unmodified rubella virus were challenged by intranasal inoculation with natural rubella virus. In a typical experiment a dose of 5000 or more $TCID_{50}$ of natural virus constituted the challenge. Fourteen of 25 previously vaccinated monkeys and four-fold or greater increases in HAI antibody while only 2 of 16 monkeys previously infected with unmodified virus showed booster responses. Virus was isolated on rare occasions from respiratory tract swabs and in one instance from an explant culture prepared from lymph nodes. This is in marked contrast to the 100% virus recovery from swabs and explants cultures of animals with no previous rubella immunity.

In another experiment three vaccinated animals challenged with 10 $TCID_{50}$ of virus did not have a booster response, while two of three animals challenged with 100 $TCID_{50}$ did have booster responses. The level of antibody at the time of challenge may be important in determining the frequency of reinfection. The vaccine group, for example, had a pre-challenge GMT of 1:161, while the group immunized with unmodified virus had a titer of 1:584.

Significance to Bio-Medical Research and the Program of the Division:

All work pertains to problems concerned with the prevention of rubella and the control of rubella vaccine.

Proposed Course of Project:

This project will be continued.

Honors and Awards:

The Max Weinstein Award for Outstanding Scientific Contributions to the Field of Cerebral Palsy. 1968 (Dr. Meyer and Dr. Parkman)

U.S. Department of Health, Education and Welfare Distinguished Service Award (Dr. Parkman)

U.S. Public Health Service Distinguished Service Medal (Dr. Meyer)

Publications:

Meyer, H.M., Jr.: Control of Viral Diseases: Problems & Prospects Viewed in Perspective. J. of Pediatrics. 73: 653-675, Nov., 1968.

Meyer, H.M., Jr. and Parkman, P.D.: Development of Live Attenuated Rubella Virus Vaccines. XII International Congress of Pediatrics, Mexico City, Dec. 1-7, 1968; Conference Proceedings vol. 2, pp. 399-406, Impresiones Modernas, Mexico City, 1968.

Parkman, P.D. and Meyer, H.M., Jr.: Prospects for a Rubella Virus Vaccine. Progress in Medical Virology, in press.

Meyer, H.M., Jr.: The Control of Rubella and Other Virus Infections in the Prevention of Mental Retardation. Mental Retard., in press.

Parkman, P.D., Meyer, H.M., Jr. and Hopps, H.E.: Production and Laboratory Testing of Experimental Live Rubella Virus Vaccine. International Symposium on Rubella Vaccines, London 1968; Symp. Series Immunobiol. Standard., vol. 11, pp. 261-270, Karger, Basel/New York, 1969.

Meyer, H.M., Jr., Parkman, P.D. and Hopps, H.E.: Clinical Characteristics of Experimental Infections Induced by Various Attenuated Rubella Vaccines, International Symposium on Rubella Vaccines, London 1968; Symp. Series Immunobiol. Standard., vol. 11, pp. 277-284, Karger, Basel/New York, 1969.

Parkman, P.D., Hopps, H.E. and Meyer, H.M.,Jr.: Rubella Virus: Isolation, Characterization and Laboratory Diagnosis, International Conference on Rubella Immunization, Bethesda, Maryland, Feb. 18-20, 1969, Am. J. Dis. Child., in press.

Meyer, H.M., Jr., Parkman, P.D., Hobbins, T.E., Larson, H.E., Davis, W.J., Simsarian, J.P. and Hopps, H.E.: Attenuated Rubella Viruses: Laboratory and Clinical Characteristics, International Conference on Rubella Immunization, Bethesda, Maryland, Feb. 18-20, 1969, Am. J. Dis. Child., in press.

Hopps, H.E., Parkman, P.D. and Meyer, H.M., Jr.: Laboratory Testing in Rubella Vaccine Control, International Conference on Rubella Immunization, Bethesda, Maryland, Feb. 18-20, 1969, Am. J. Dis. Child., in press.

Serial No. DBS 127
1. Laboratory of Viral Immunology
2. Section on Viral Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of viruses associated with infectious mononucleosis and human lymphoma

Previous Serial Number: DBS 127

Principal Investigator: Paul Gerber, Ph.D.

Other Investigators: John Branch, M.D.
Nicholas Rogentine, M.D.
John Walsh, M.D.
Edith N. Rosenblum, B.S.

Cooperating Units: Blood Bank Department, Clinical Center
Immunology Branch, NCI

Man Years:

Total	2.0
Professional	1.0
Other	1.0

Project Description:

A herpes-like virus, generally termed Epstein-Barr Virus (EBV) has been detected by a number of investigators in cultures of lymphoid cells derived from Burkitt lymphoma, leukemia patients, infectious mononucleosis patients and from normal donors. During the past year the possible causal relationship of EBV to infectious mononucleosis has been demonstrated mainly by serological methods.

The current efforts were aimed at:

(1) comparing the sensitivity of various serological methods for the detection of EBV antibodies.

(2) demonstrating the transmission of EBV infection in non-human primates and man.

(3) establishing the true incidence of infectious mononucleosis in a susceptible population group.

(4) detecting changes of surface antigens in transformed human leukocytes harboring EBV.

Major Findings:

(1) The results of a collaborative study with several investigators to compare the specificity and sensitivity of various serological methods in the detection of EBV antibodies revealed excellent correlation among complement-fixation, immuno-diffusion, immuno-fluorescence and antibody coating tests. Of these, the immuno-fluorescence and complement-fixation test were the most sensitive.

(2) Attempts to transmit infectious mononucleosis to selected rhesus monkeys and marmosets which were free of detectable EBV antibodies were unsuccessful, however, transmission of EBV by blood transfusion and development of clinical illness diagnosed as infectious mononucleosis was demonstrated in 1 of 5 post-operative patients.

(3) No significant changes in leukocyte antigens (HLA) were detected when both fresh lymphocytes and those maintained in long term culture of the same donor were compared.

Significance to Bio-medical Research and the Program of the Division:

Studies of infectious diseases and viral oncogenicity are an integral part of the mission of DBS.

Proposed Course of Project: This project is continuing.

Publications:

Paul Gerber, John W. Branch and Edith N. Rosenblum: Attempts to Transmit Infectious Mononucleosis to Rhesus Monkeys and Marmosets and to Isolate Herpes-like Virus. Proc. Soc. Exp. Biol. Med. 130: 14-19, 1969.

Paul Gerber, John W. Walsh, Edith N. Rosenblum and Robert H. Purcell: Association of EB-virus infection with the post-perfusion syndrom Lancet 1: 593-596, 1969.

Nicholas G. Rogentine and Paul Gerber: HLA Antigens of human lymphoid cells in Long-term culture. Transplantation (in press).

Report from Immunology sub-group, special virus-cancer program. Results of a comparison of serological tests used to detect human antibodies to a herpes virus (HLV). J. Nat. Cancer Inst. 42: 623-631, 1969.

Paul P. Carbone, Costan W. Berard, John M. Bennett, John L. Ziegler, Martin H. Cohen and Paul Gerber: Burkitt's Tumor. Arch. Int. Med. (in press).

Serial No. DBS 47
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigations concerning the smallpox group of viruses and smallpox vaccines

Previous Serial Number: DBS 47

Principal Investigator: Lewellys F. Barker, M.D.

Other Investigators: David P. Stevens, M.D.
Daniel E. Thor, M.D.
Sheldon M. Wolff, M.D.
Harry M. Meyer, Jr., M.D.
Hope E. Hopps, M.S.

Cooperating Units: Laboratory of Virology and Rickettsiology, DBS
Laboratory of Clinical Investigation, NIAID

Man Years:

Total:	2.5
Professional:	1.6
Other:	0.9

Project Description: Part I. Study of immunity to vaccinia virus in experimental animals.

The rhesus monkey has been shown to provide a useful laboratory model for studying smallpox vaccination. In an effort to develop methods for distinguishing between the pathogenicity of vaccinia strains in the laboratory, we have compared the dermal reactivity of 4 strains of vaccinia in monkeys and rabbits. The strains included the DBS potency test reference lot #2, which is a calf lymph propagated strain (CL), the same strain passaged 115 times in primary chick embryo fibroblast cultures (PCE), a commercial low passage chorioallantoic membrane produced vaccine (CAM) and the CVI-78 high egg passage strain. Injection of monkeys was subcutaneous and in rabbits the virus was inoculated intradermally. The CVI-78 strain was the most reactive, producing dermal necrosis in monkeys at a dose of 2×10^3 $TCID_{50}$ and in rabbits at a dose of 10^2 $TCID_{50}$. The CAM vaccine produced necrotic lesions in monkeys at a dose 1.6×10^7 $TCID_{50}$ and in rabbits at a dose of 10^4 $TCID_{50}$. The CL strain produced a small necrotic reaction in one monkey at a dose of 3×10^7 $TCID_{50}$ and erythema and induration, but no necrosis, in rabbits at the highest dose tested, 10^6 $TCID_{50}$. After passage of the CL virus 115 times in PCE fibroblasts, it produced no necrosis in either monkeys or rabbits at the highest doses tested. Thus on the basis of pathogenicity in monkeys and rabbits, these 4 strains of

vaccinia could be classified as highly reactive (CVI-78), intermediate (CAM and CL) and attenuated (PCE).

Efforts to distinguish between vaccinia strains by their capacity to induce interferon synthesis in a variety of cell types have been unsuccessful to date.

Project Description: Part II. Clinical Studies.

A study of the correlation between humoral and cellular immunity and response to revaccination was conducted in the Clinical Center in healthy volunteers. Ten of 12 volunteers had major reactions to revaccination; these individuals demonstrated: (1) significant increases in lymphocyte thymidine incorporation compared to pre-immunization levels of reactivity, and (2) the appearance of antigen-specific migration inhibitory factor production. Significant increases in plaque neutralizing antibody titers accompanied major reactions in most instances, but there was a wide range of pre-existing neutralizing antibody titers prior to revaccination. Hemagglutination-inhibiting antibodies were of no value in judging revaccination reactions.

Significance to Bio-medical Research and the Program of the Division:

All of this work directly pertains to research problems in the prevention of smallpox and control smallpox vaccine.

Proposed Course of Project: This project will be continued.

Honors and Awards:

Dr. Meyer and Dr. Barker were invited to lecture at the NCDC at the 6th Annual Immunization Conference, March 11-14, 1969.

Publications:

Barker, L.F. and Meyer, H.M., Jr.: Further attenuated vaccinia virus: a possible alternative for primary immunization. Proceedings of the sixth Annual Immunization Conference, NCDC, Atlanta, Georgia, March 11-13, 1969, in press.

Serial No. DBS 53
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Study and diagnosis of heritable metabolic disease by use of tissue culture.

Previous Serial Number: DBS 53

Principal Investigator: B. William Uhlendorf, Ph.D.

Other Investigators:
S. Harvey Mudd, M.D.
Howard R. Sloan, M.D., Ph.D.
John P. Blass, M.D.
Wilfred Fujimoto, M.D.
Joseph C. Frantantoni, M.D.
Elisabeth F. Neufeld, Ph.D.
Allen Lapey, M.D.
Paul A. di Sant-Agnese, M.D.
Roscoe O. Brady, Jr., M.D.
Edwin H. Kolodny, M.D.
Seymour Kaufman, Ph.D.
Cecil B. Jacobson, M.D.

Cooperating Units:
Laboratory of General and Comparative Biochemistry, NIMH
Molecular Disease Branch, NHI
Arthritis and Rheumatism Branch, NIAMD
Laboratory of Biochemistry and Metabolism, NIAMD
Pediatric Metabolism Branch, NIAMD
Laboratory of Neurochemistry, NINDB
Laboratory of Neurochemistry, NIMH
Department of OB/GYN, George Washington University Medical Center

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description: The concept which is the basis of the subprojects listed below is that a heritable metabolic disorder of an individual's metabolism may be reproduced in cultures of his cells.

Methods which are standard in tissue culture, histochemistry, chromatography, radioautography, chemical analysis, enzyme assay and radioactive tracer studies were used.

Objectives and Major Findings:

A. To further characterize the growth of human fetal cells obtained by amniocentesis. --- An additional 51 samples of fresh, sterile amniotic fluid were received. Of these 47 were successfully cultured, 38 in sufficient quantity for enzyme assay. Twenty-four of the cell lines were preserved in liquid nitrogen.

B. For the purpose of establishing bases for the in utero diagnosis of various inborn errors of metabolism, the activities of several enzymes (or the accumulation of a product) in amniotic fluid cell cultures were determined.

1) On the basis of the presence of cystathionine synthase in cell cultures from a sample of amniotic fluid from a woman who had previously given birth to two homocystinuric children the prediction was made (correctly) that the pregnancy would result in a normal individual.

2) In an amniotic fluid cell culture from a woman known to be a carrier for x-linked uric aciduria (Lesch-Nyhan syndrome) we found two types of clones. On type exhibited hypoxanthine-guanine phosphoribosyltransferase activity and the other did not. The cells had the female karyotype. The prediciton was made (correctly) that the pregnancy would result in a normal female heterozygous for the enzyme deficiency.

3) On the basis of metachomatic staining of cultured amniotic fluid cells and their kinetics of incorporation of radioactive sulfate into mucopolysaccharides we made the prenatal diagnosis of the Hurler syndrome and the Hunter syndrome for two women who had previously borne children with these disorders. In the one case the diagnosis was verified by laboratory studies on the infant and in the other case the pregnancy was therapeutically interrupted.

4) Normal amniotic fluid cell cultures were consistently found to have activities of the following enzymes; cystathionase, methylmalonyl-CoA-isomerase, sphingomyelinase, G_{M1}-β-galactosidase, sulfatide sulfatase, glucocerebrosidase, α-ketoisocaproate decarboxylase and phytanate α-hydroxylase; thus providing the biochemical basis for the intrauterine diagnosis of; cystathioninuria, methylmalonic aciduria, Niemann-Pick disease (types A & B), generalized (G_{M1}) gangliosidosis, metachromatic leucodystrophy, Gaucher's disease, maple syrup urine disease, and Refsum's disease.

5) We have demonstrated the specific accumulation of G_{M2}-ganglioside (Tay-Sach's ganglioside) in cell cultures from skin biopsies of patients with Tay-Sach's disease and the absence of such accumulation in control cultures. Based on the accumulation of this ganglioside in cells cultured from umbilical cord the biochemical diagnosis (later verified by clinical diagnosis) was made for a 2 month old child. Normal amniotic fluid cultures do not contain detectable G_{M2}-ganglioside suggesting the possibility of in utero diagnosis of Tay-Sach's disease.

6) A histochemical procedure for β-galactosidase has been applied to cell cultures grown on cover slips. There is a specific lack of reaction product (bis-indigo) in cells cultured from a skin biopsy of a patient with gen-

eralized gangliosidosis indicating the lack of β-galactosidase activity in his cultured cells. Control amniotic fluid cells had histochemically demonstrable enzyme activity. An amniotic fluid cell culture from a woman who had previously borne a child with generalized gangliosidosis also had activity indicating that the pregnancy will result in a normal child.

7) There appears to be a small but definite activity of phenylalanine hydroxylase in a control skin culture and a control amniotic fluid cell culture. This enzyme is lacking in the liver of patients with phenylketonuria (PKU). If the conditions of cell culture and/or enzyme assay can be altered in such a way that the demonstration of phenylalanine hydroxylase activity is unequivocal, the *in utero* diagnosis of PKU should be possible.

C. The nature of the underlying metabolic defect in several metabolic diseases is being studied by use of skin fibroblast cultures.

1) A new metabolic disorder in which a derangement in vitamin B_{12} metaboliam leads to homocystinemia, cystathionemia and methylmalonic aciduria is being studied in cell cultures derived from a skin biopsy of an infant who subsequently died of the disorder. Whereas control cultures grew equally well in the presence of methionine or homocystine + B_{12}, the mutant culture will not grow in the absence of methionine. It also lacks the ability to oxidize methylmalonic acid unless supplemented with very high levels of hydroxy-B_{12}.

2) An inherited defect in pyruvic decarboxylase has been demonstrated in a boy with an intermittent movement disorder associated with stress. Cell cultures from the patient have a greatly impaired ability to oxidize pyruvic acid.

3) A study of cystic fibrosis using the "tissue culture approach" has been initiated. Efforts have been and will be along three main avenues: (a) chemical analysis of large quantities of cells produced in roller bottle cultures, (b) histochemistry, electron microscopy and electron probe studies of cells grown on cover slips, and (c) studies employing radioactively labeled substrates.

Significance to Bio-medical Research and the Program of the Division:

Human diploid cell culture is a powerful tool for the early intrauterine diagnosis of heritable metabolic diseases and for studies of their underlying biochemical defects.

Proposed Course of Project: This is a continuing project.

Honors and Awards:

Invited to participate at the annual spring session of the American Academy of Pediatrics, April 21-23, 1969, Boston, Massachusetts.

Publications:

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Fujimoto, W.Y., Seegmiller, J.E., Uhlendorf, B.W. and Jacobson, C.B.: Biochemical diagnosis of an x-linked disease in utero. Lancet 2: 511-512, 1968.

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Serial No. DBS 137
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigations into the etiologic agent of viral hepatitis.

Previous Serial Number: 137

Principal Investigators: Lewellys F. Barker, M.D.
David P. Stevens, M.D.

Other Investigators: Kendall O. Smith, M.D.
Ruth L. Kirschstein, M.D.
Douglas E. Lorenz, Ph.D.
N. Raphael Shulman, M.D.
Richard J. Hirschman, M.D.
Colonel Robert Fike, M.D.
Colonel Eugene D. Shaw, Ph.D.
Warren D. Gehle, B.S.
Eva R. Brown, B.S.
Barbara C. Meyer, M.A.
Hope E. Hopps, M.S.
Frank Ruddle, Ph.D.

Cooperating Units: Department of Biology, Yale University
Surgery Branch, National Cancer Institute, NIH
Department of Internal Medicine, DeWitt Army Hospital, Fort Belvoir, Virginia
Hematology Branch, National Institute of Arthritis and Metabolic Diseases, NIH
Laboratory of Pathology, DBS, NIH
Laboratory of Biophysics and Biochemistry, DBS, NIH
Virology Section, Armed Forces Institute of Pathology

Man Years:

Total:	3.2
Professional:	1.9
Other:	1.3

Project Description: Part I. Isolation attempts.

Continuous lymphoblastoid tissue cultures derived from peripheral blood of patients with infectious hepatitis and normal healthy donors were established in an effort to provide tissue culture material which might serve as host to the agents of infectious hepatitis *in vitro*. These cell lines have been studied for presence of infectious agents using standard tissue culture, fluorescent antibody, electron microscopic and cytogenetic techniques.

Project Description: Part II. Identification of candidate agents.

Studies have continued on several "candidate agents" -- uncharacterized infectious agents obtained from patients with viral hepatitis -- in an effort to identify them and demonstrate their relevance to this disease.

Project Description: Part III. Prospective Survey for Serum Hepatitis.

Surgical patients of the Surgery Branch, National Cancer Institute, who received blood transfusions have been followed prospectively to determine evidence of viral hepatitis in this group. Serial serum specimens have been obtained for serum enzyme determinations to determine the presence of liver disease. Changes have been sought in levels of antibody against viral agents which might play an etiologic role in hepatitis.

Project Description: Part IV. Evaluation of the relationship of Australia-antigen associated virus-like particles to viral hepatitis

Characterization and immunologic evaluation of the virus-like particles associated with the Australia antigen described by Blumberg have been conducted.

Objectives: The goal of these studies is the isolation and identification of infectious agents which are etiologic in viral hepatitis.

Methods Employed: Standard serologic, tissue culture, fluorescent antibody, electron microscopic and cytogenetic techniques are used throughout the project.

Major Findings: Continuous lymphoblastoid tissue cultures were established from 21 of 35 (60%) patients who were part of an epidemic of acute infectious hepatitis at Fort Belvoir, Virginia. Cultures were also established from 3 of 16 (19%) healthy control donors. This demonstrated that cultures of this type are more readily established from peripheral blood of patients with infectious hepatitis than from healthy donors. Other studies of these cells, however, have not demonstrated cytopathic, hemadsorbing, interfering or fluorescing agents which could be related to infectious hepatitis. Cytogenetic studies performed by Dr. Frank Ruddle, Yale University, demonstrated normal diploid karyotypes in all lines. Preliminary electron microscopic studies, performed under contract by Melpar, Inc., Falls Church, Virginia, have not demonstrated any suspect particles in these cell lines.

Forty-three patients who received multiple blood transfusions on the Surgery Branch, National Cancer Institute, were followed prospectively for evidence of serum hepatitis. Eight developed enzyme changes consistent fith that disease. Because of reports by others suggesting that cytomegalovirus (CMV) may play an etiologic role in certain forms of hepatitis following blood transfusion, complement fixing antibodies against CMV were evaluated in this same group of transfused patients. While a four-fold or greater rise in antibody titer was observed in 13 of 41 patients (32%) studied and CMV was isolated from the urine of one patient, these findings could not be correlated with any clinical illness including hepatitis, fever, or mononucleosis.

Paired sera from patients with viral hepatitis were evaluated for plaque-reducing antibodies against the "A-2" agent, an enterovirus-like agent which is under study by Colonel E. D. Shaw of the Armed Forces Institute of Pathology. Absence of significant changes in titer of antibodies against this agent indicate that it is probably not related to the agent of serum or infectious hepatitis.

The Australia antigen and associated virus-like particles have been found in increased incidence in sera of patients with infectious and serum hepatitis. Physical characteristics and fine structure of the particles are consistent with a viral identity. Serologic studies and evaluation of the possibility that the particles are infectious in cell cultures and marmosets are in progress.

Significance to Bio-medical Research and the Program of the Division:

All of this work is pertinent to the goals of the DBS.

Honors and Awards: None

Publications: None

Serial No. DBS 46

1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigations concerning measles virus and vaccine

Previous Serial Number: DBS 46

Principal Investigator: Barbara C. Meyer, M.S.

Other Investigators: Hope E. Hopps, M.S.
Robert D. Douglas
Harry M. Meyer, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	0.6
Other:	0.6

Project Description: New measles potency assay system.

As mentioned in last year's report, studies were initiated to determine whether the Vero line of Cercopethecus kidney cells might be used for potency assay of live, attenuated measles virus vaccine. In comparative titrations using tube cultures of BS-C-1 and Vero cells, the titration endpoints were 0.5 logs higher in Vero cells than in BS-C-1. In attempting to adapt the Vero cell system to a microtiter assay, Vero cell suspension (2×10^4 cells) was placed in the wells of Limbro microtiter tissue culture plates. The virus, diluted in 0.5 log steps was than added. The cultures were incubated at 35°C in a CO_2 incubator. Final CPE readings were read after 14 days. The results indicate that this system is equal in sensitivity to assays performed in BS-C-1 on Vero tube cultures. At the present time, all vaccine potency assays are run by the Vero microtiter tissue culture procedure.

Significance to Bio-medical Research and the Program of the Division:

This work relates directly to the measles vaccine control program.

Proposed Course of Project:

As newer and improved methods for viral assay are developed they will be tested in comparison with the systems currently in use in order that the most efficient and sensitive tests can be adapted for vaccine potency assay.

Honors and Awards: None

Publications: None

Serial No. DBS 52
1. Laboratory of Viral Immunology
2. Section on Viral Genetics
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on Simian Virus$_{40}$.

Previous Serial Number: 52

Principal Investigator: Paul Gerber, Ph.D.

Other Investigators: Edith N. Rosenblum, B.S.

Cooperating Units: None

No work was done on this project during fiscal year 1968-1969

Serial No. DBS 126
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigations concerning combined, live virus vaccine.

Previous Serial Number: DBS 126

Principal Investigator: Harry M. Meyer, Jr., M.D.

Other Investigators: Hope E. Hopps, M.S.
Lewellys F. Barker, M.D.
Barbara C. Meyer, M.S.
Robert D. Douglas

Cooperating Units: None

No work was done on this project during fiscal year 1968-1969.

Publications:

Harry M. Meyer, Jr.: The Control of Viral Diseases: Vol 73, No. 5 pp. 653-675

Serial No. DBS 171
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigations concerning Cytomegaloviruses

Principal Investigator: William J. Davis, M.D.

Other Investigators: Paul D. Parkman, M.D.
Laura Baridon, B.S.

Cooperating Units: None

Man Years:

Total:	1.9
Professional:	1.5
Other:	0.4

Project Description: Isolation, characterization and serologic studies of cytomegaloviruses.

The discovery in 1960 of simian virus 40, a common latent virus contaminant of rhesus monkeys and cell cultures derived from the renal tissues of infected monkeys, and the development of the RIF test by Rubin, demonstrating the widespread distribution of avian leucosis virus in chicken flocks and in the embryos from these flocks greatly increased the interest in surveillance of tissues used for adventitious agents. As our experience has grown it becomes evident that many apparently healthy tissues can harbor latent or slow growing viruses not readily detected by ordinary cell culture tests. The cytomegaloviruses (CMV) a group of agents commonly found in many animal species, fall into this category of viruses. Experience indicates that they are frequently present in primary African green monkey kidney cell cultures (GMK). Cytomegalovirus isolation attempts from randomly selected lots of GMK cells showed nine of ten to contain simian CMV. Co-cultivation of the GMK cell suspension with WI-38 cells yielded CMV isolates from all of the nine positive cell lots, and in each instance provided the earliest indication of CMV contamination; definite evidence of typical CMV cytopathic effect (CPE) was first detected from 17 to 26 days after initiation of these mixed cultures. CMV was also readily isolated from contaminated cell lots by passage of cells and culture fluids into WI-38 cells. Positive isolations were made from supernatant fluids of eight of the nine contaminated cell lots examined and from the combined cells and supernatant fluids of six subpassages of supernatant fluids to the other types of cultures used, including HEK, RK, RhMk and GMK, did not detect CMV from any of the lots; prolonged observation of cultures from the cell lots under study were positive for CMV CPE in four of ten instances in which it was possible to observe cultures for 28 to 50 days. Sera from eight of the nine monkeys from whom GMK cultures were prepared were positive for CMV complement-fixing (CF) antibodies. The one

uncontaminated GMK cell lot was derived from a monkey devoid of CF antibody. This finding suggested that the CMV CF test might provide a method for screening suitable animals for cell culture production.

In an attempt to learn more about the epidemiology of this virus in monkeys, studies were performed using sera collected from groups of animals in the jungle, and from quarantined groups of animals after various periods of time in captivity. Sera were tested for CMV antibodies with virus strains recovered from African green or rhesus monkeys in WI-38 cells. Sera from each species reacted to higher titer with the homologous virus. It was apparent that a high percentage of animals procured through the usual channels and quarantined for 6-8 weeks in holding areas in this country had been infected with cytomegaloviruses. Thus, in two different groups of green monkeys, 10 of 10 and 9 of 10 animals had serologic evidence for prior infection with CMV. More complete seroepidemiological information was obtained on CMV infections in rhesus monkeys. Sera from 27 such animals drawn at the time of capture in the jungle were available for study; only 1 of these 27 animals was positive for cytomegalovirus CF antibody. In contrast, in rhesus monkeys from two different quarantine areas in this country, 11 of 15 and 9 of 9 respectively were positive for CF antibodies. The four seronegative animals in the first group were caged apart from other young monkeys and were bled at intervals over a 5 month period. During this observation period 3 of the 4 developed CF antibodies.

Thus it appears that the epidemiology of CMV infections in monkeys resembles that of SV-40. Only a small percentage of young animals trapped in the jungle have been infected, but following the period of days or weeks when animals are held in large groups prior to distribution to laboratories, and during the quarantine period in this country, the epidemiologic circumstances favor the spread of CMV until essentially 100% of animals become infected. It would seem possible, on the basis of this experience to procure seronegative animals and through special handling procedures such as those described for obtaining measles susceptible monkeys (Meyer et al, Ecology of Measles in Monkeys, Am. J. Dis. Child. 103: 307-313, 1962) to obtain animals without CMV infection. Such procedures would be useful for the preparing of CMV-free cell culture and for the study of experimental CMV infections.

Significance to Bio-medical Research and the Program of the Division:

The characterization of agents which may be of importance to vaccine safety is an important mission of the Division.

Proposed Course of Project:

Studies concerning relationship between simian cytomegalovirus strains will be continued.

Honors and Awards: None

Publications: None

Serial No. DBS 172
1. Laboratory of Viral Immunology
2. Section on General Virology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigations concerning respiratory syncytial virus.

Previous Serial Number: None

Principal Investigator: Thomas E. Hobbins, M.D.

Other Investigators: Joan C. Enterline, A.B.

Man Years:

Total:	0.5
Professional:	0.5
Other:	- -

Project Description: Search for a hemagglutinin associated with RS virus.

The successful demonstration of hemagglutination (HA) by rubella virus produced in several types of cell cultures using inhibitor-free culture medium and concentration by density gradient ultracentrifugation led us to examine a respiratory syncytial virus using similar techniques. This agent has several characteristics suggesting that it may be related to the paramyxoviruses, including an RNA core, ether sensitivity and morphologic appearance, with the presence of capsular "spikes".

RS virus was grown in Hep 2 cells maintained with Eagles minimum essential medium with 2% kaolin treated fetal bovine serum. Supernatant fluid harvests were banded in preformed, linear cesium chloride (CsCl) gradients after sonic treatment and celite filtration. A single, visible band was seen in each gradient; infectivity titrations showed that virus was concentrated in the area of the observed band.

Fractions from such gradients were used as antigens in tests for hemagglutination performed at 4°C in DGV containing 0.2% bovine plasma albumin. Several red blood cell types were used including those from sheep, human guinea pig, monkey, baby chick, turkey, goose, Pekin duck and mallard duck sources. No specific hemagglutination was observed with any of these preparations.

Significance to Bio-medical Research and the Program of the Division:

The development of new serologic tests is important to the DBS control testing program.

Proposed Course of Project: This project is continuing.

Honors and Awards: None

Publications: None

Serial No. DBS 48
1. Laboratory of Viral Immunology
2. Section on Immunology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigations Concerning Mumps Virus and Vaccine.

Previous Serial Number: DBS 48

Principal Investigator: Hope E. Hopps, M.S.

Other Investigators: James P. Simsarian, M.D.
Robert D. Douglas
Barbara C. Meyer, M.S.
Eva R. Brown, B.S.
Harry M. Meyer, Jr., M.D.

Cooperating Units: None

Man Years:

Total:	2.3
Professional:	1.9
Other:	0.4

Project Description: Part I. Studies directed toward the standardization of serologic tests for mumps.

Since the complement-fixation (CF) and hemagglutination-inhibition tests (HI) for mumps antibody are not sensitive indicators of low levels of antibody, efforts have continued to devise a sensitive plaque neutralization test to be used in screening for mumps susceptibility prior to vaccination. Such a test would also be useful in following the duration of mumps antibody over a period of years. Such studies are of particular importance in assessing the efficacy of live attenuated mumps virus vaccine.

During the past year we have published a report of investigation describing a plaque neutralization test carried out in MA-104 (stable embryonic rhesus kidney cells). After *in vitro* incubation of test serum and virus, the cell monolayers are inoculated and overlayed with agar. Plaques are counted after 7-8 days and antibody titer is expressed as a 50% endpoint based on number of plaques formed.

The only drawback to the present test is in the size of the plaques formed; the average diameter is 0.5 to .75 mm and they are often difficult to count. More recently, experiments have been carried out with the Vero (stable Cercopithecus kidney) cell line and this latter system may have certain advantages over the MA-104 cell system. The plaques formed are somewhat larger (1-2 mm) with discrete borders which facilitatesthe counting process.

Project Description: Part II. Clinical studies with natural and attenuated mumps infection.

Using the Vero cell plaque neutralization test, studies are now in progress to determine the stability of antibody in children at the Arkansas Children's Colony who received live attenuated Jeryl Lynn strain vaccine two years ago. After one year, the levels of HI and plaque neutralizing antibody remained unchanged. A slightly less than 2-fold decrease in soluble CF antibody was noted.

Project Description: Part III. The development of a microtiter tissue culture potency assay for live attenuated mumps vaccine.

A suitable mumps vaccine potency assay employing BS-C-1 tube cultures was developed in this laboratory. Potency endpoints were read by both CPE and guinea pig erythrocyte hemadsorption. However, a less cumbersome system was sought and during the past 7 months a new potency assay procedure was developed. Here, Vero cells are inoculated into Linbro microtiter tissue culture plates (approximately 2 x 10^4 cells per well). Diluted virus is then added and the plates are held for 7 days at 35°C in a CO_2 incubator. Comparative titrations in the 2 systems indicate the Vero cells to be of comparable sensitivity to BS-C-1. The new test is simple, accurate and rapid; henceforth, all mumps vaccine potency assays will be performed using this microtiter technique.

Project Description: Part IV. Animal studies with natural and attenuated strains of mumps virus.

Studies concerning the induction of obstruction hydrocephalus in suckling hamsters by IC inoculation of mumps virus have continued. Several experiments with both low passage and attenuated (Jeryl Lynn) mumps viruses were carried out. In one experiment, 4 groups of 2 day old animals were used. Forty-nine hamsters received 200 $TCID_{50}$ of virulent virus and 40 received 200 $TCID_{50}$ of the Jeryl Lynn strain; 37 received neutralized virus and 37 served as uninoculated controls. During the first 5 weeks after inoculation death occurred in 23 of the animals receiving the Jeryl Lynn strain and 21 of those receiving low passage virus. In those animals dying after day 14, all had developed gross hydrocephalus.

When either virus was administered IC, IM or IP, no hydrocephalus was observed; nor did maternal mumps infection during gestation produce any evidence of infection or hydrocephalus in newborn hamsters.

Proposed Course of Project:

Studies concerning the effect of low and high passage mumps virus in newborn monkeys are now in progress.

Significance to Bio-medical Research and the Program of the Division:

All of this work is pertinent to the goals of the DBS.

Honors and Awards: None

Publications:

Ennis, F.A., Douglas, R.D., Hopps, H.E., Meyer, H.M., Jr., Brown, E.R., Hobbins, T.E. and Biehusen, F.C.: Clinical Studies with Virulent and Attenuated Mumps Viruses. Am. J. of Epid. 89: 176-183, 1968.

Ennis, F.A., Douglas, R.D., Stewart, G.L., Hopps, H.E. and Meyer, H.M., Jr.: A Plaque Neutralization Test for Determining Mumps. Proc. Soc. Exp. Biol. Med. 129: 896-899, 1968.

Ennis, F.E., Hopps, H.E., Douglas, R.D. and Meyer, H.M., Jr.: Hydrocephalus in Hamsters: Induction by Natural and Attenuated Mumps Viruses. 119: 75-79, 1969. J. Infect. Dis.

LVR

Annual Report of the
Laboratory of Virology and Rickettsiology
Division of Biologocs Standards
July 1, 1968 through June 30, 1969

A. ADMINISTRATIVE ASPECTS

Introduction. Notable organizational and personnel changes have occurred in this Laboratory. New emphasis is being placed on the development of a program which would coordinate straightforward control with control-related research responsibilities.

Mission Statement. The Laboratory encompasses a broad program of research concerning the prevention and treatment of viral and rickettsial infections in man. Specific emphasis is placed upon problems as they relate to the mission of DBS, such as problems concerned with vaccine safety, potency, efficacy and standardization. Constant liaison with the Division's other Laboratories achieves a balance in the harmonious execution of the DBS control and research responsibilities.

Personnel Changes. Key personnel changes have occurred. Notable among these were the retirments on 31 October 1968 of Alexis Shelokov, M.D., Chief of the Laboratory, and C. P. Li,M.D., Chief of the Virus Biology Section. Jose G. Canchola, M.D., Head of the Respirovirus Unit, resigned in December 1968, and Ruth C. Dunlap, Ph.D., Head of the Virus Morphology Unit, transferred to the Division's Laboratory of Biophysics and Biochemistry in January 1969.

Additions to the senior professional staff were: (1) Daniel E. Thor, M.D., Ph.D. (July 1968) who will continue his studies in the field of immunobiology; (2) Thomas C. O'Brien, Ph.D., (June 1969) who will become the key professional in our Respirovirus Unit; and (3) Heinz P. Schumacher, M.D. (January 1969), International Postdoctoral Research Fellow from Germany, who is working in LVR's Viral Pathogenesis Unit.

Roderick Murray, M.D., served as Acting Chief of the Laboratory from 1 November 1968 to 21 January 1969 at which time Nicola M. Tauraso, M.D., Head of LVR's Immunoserology Unit, became Acting Chief.

Organizational Changes. On 28 February 1969 the Laboratory was reorganized to reflect the personnel changes and the new emphasis upon the Laboratory's program. The Respiratory Viruses and Virus Biology Sections were abolished. The name of the Basic Virology and Rickettsiology Section was changed to the Virology and Rickettsiology Section, and the Tissue Culture Section was renamed the Cell Biology Section. As a result of

these changes, the Laboratory of Virology and Rickettsiology is now composed of an Office of the Chief, the Virology and Rickettsiology Section, and the Cell Biology Section.

The Virology and Rickettsiology Section is responsible for conducting basic and applied research concerning the prevention and treatment of viral and rickettsial infections in man, with specific emphasis on problems as they relate to the mission of DBS, such as problems concerned with vaccine safety, potency, efficacy and standardization. This Section is composed of the following 7 Units: Immunoserology, Avian Leukosis, Immunofluorescence, Respirovirus, Arbovirus, Rickettsial and Viral Pathogenesis. Nicola M. Tauraso, M.D., is Chief of this Section.

The Cell Biology Section is responsible for maintaining a facility to provide cell cultures to DBS investigators and for conducting an ongoing research program concerned with problems which might arise from the use of cell cultures for preparing virus and rickettsial vaccines. This Section is composed of the Cell Production and Cell Research Units. Henry C. Orr, Ph.D., is Acting Chief of this Section.

B. RESEARCH ACTIVITIES

Influenza Vaccine. A major portion of the Laboratory is devoted to the execution of the Division's responsibility with regard to influenza vaccine. A constant effort is required: (1) to maintain a competent Respirovirus Unit for the study of influenza viruses and other viral agents etiologically associated with respiratory tract infections of man; (2) to maintain a reference collection of infectious viral antigens and antibody reagents of influenza and other respiroviruses including those of potential importance as vaccine constituents; (3) to review and analyze manufacturers' protocols and to perform potency tests on current influenza vaccines; and (4) to improve serologic techniques for immunogenic potency testing of influenza vaccines.

Heretofore, the CCA test was considered reliable as a measure of the antigen content of influenza vaccines because the test was not standardized. During this past year, great strides have been made to make the CCA test reproducible not only within a laboratory but also between different laboratories. This was especially helpful to us in carrying out our control mission during this season's epidemic caused by the A2/Hong Kong/68 variant.

DBS was able to make some of its vaccine (ordinarily used for the purposes of control) available to competent investigators early enough so that major volunteer studies were initiated. Our Laboratory was directly involved in 2 different studies which were completed prior to the major epidemic wave.

Yellow Fever Vaccine. The DBS yellow fever project was established in April 1966 as a coordinated research program involving several laboratories in the Division. Scientific profits have been realized from this team approach to problem solving.

An avian leukosis virus (ALV)-free primary yellow fever vaccine has been developed by ridding the 17D virus vaccine of its ALV contaminant. By differential filtration through Millipore membrane filters of different pore size, the smaller yellow fever virus was separated from its larger contaminant by physical means. The ALV-free candidate primary and secondary seed lots were indistinguishable from corresponding ALV-contaminated lots with respect to: (1) potency as measured by titration in newborn and weanling mice and in the MA-104 plaque system; (2) degree of viscerotropism as measured by viremia in monkeys; (3) neurotropism as determined by the monkey neurovirulence test; and (4) potency as determined by antibody response in monkeys inoculated by the intracerebral route.

Studies on immunogenicity were conducted on a total of 265 rhesus monkeys and on 180 volunteers at the Lewisburg Federal Penitentiary. The ALV-free yellow fever vaccine was indistinguishable from the currently available commercial ALV-contaminated vaccine with respect to reactions and to antibody response.

In the course of these studies a plaque neutralization test was developed; it has many practical and theoretical advantages over the standard yellow fever mouse neutralization test.

Smallpox Vaccine. A system of enzyme treatment for smallpox vaccines has been devised which reduces the extraneous debris sufficiently that a direct electron microscope particle count of the virus can be made. The direct count of virus particles provides a quick, easy quantitation which can then be used to correlate other methods of titration. Studies on aggregation-dispersion performed with smallpox vaccine virus, passed once in cell culture to eliminate calf lymph debris, indicate that aggregation increases the infective titer.

Adenovirus Vaccine. At the present time adenovirus vaccines are not licensed in the United States, and, hence, are not generally available to the public. Surprising as it may seem, little is known about the biology of human adenovirus infection. The Laboratory continues its research studies on adenoviruses with the hope that, eventually, the proper place for adenovirus vaccines in the armamentarium of respirovirus vaccines will be known.

In searching for an experimental animal our Laboratory was unsuccessful in attempts to infect rhesus monkeys with human adenovirus type 4. However, a fortuitious accident and keen investigative studies established the fact that human adenovirus type 31 caused an outbreak of coryza among chimpanzees.

Egg Vaccines. The developing chicken embryo remains one of the most versatile substrates for vaccine manufacture. In order to maintain vaccine purity, it has been necessary to survey avian flocks for potentially troublesome microbial infections and to maintain chicken flocks which are free of known viral, bacterial and mycoplasma contaminants. The Avian Leukosis and Immunofluorescence Units of this Laboratory provide the DBS with the competency to deal with potential problems in this area.

Studies on the group specific antibody of avian leukosis viruses in experimentally infected pigeons might simplify the testing for these endogenous chicken viruses. This would be advantageous both to vaccine manufacturers and DBS.

Studies have demonstrated the usefulness of substituting egg yolk antibody for serum antibody in the immunofluorescence test to study agents of all microbial groups (viruses, rickettsiae, bedsoniae, mycoplasmas, and bacteria). The capability of the immunofluorescence test to detect avian leukosis viruses from the different subgroups makes it an important adjunct to the RIF test in the safety testing of human viral vaccines of egg origin.

Studies are being performed to determine whether Marek's disease of chickens can be transmitted via the egg. The extent and significance of this potential contaminant will be evaluated with the hope of developing satisfactory methods for testing potential contamination in chicken embryos to be used for vaccine production.

Rickettsial Vaccines. As with most killed antigens, it is difficult to measure the potency of rickettsial vaccines. Emphasis was directed toward evaluating a candidate lot of inactivated typhus vaccine for use as a reference antigen. Although this reference vaccine evoked a good antibody response in man, it was considered sub-optimal as a reference on the basis of its direct complement-fixing antigen titer and marginally satisfactory with regard to its guinea pig potency titer. Future studies will be concerned with evaluating what correlation exists between the laboratory tests for potency and the immunological response evoked in man.

Additional studies concerned: (1) definition of the nutritional requirements of the intracellular parasite Rickettsia quintana, and (2) comparison of the genetic relatedness of rickettsial and other intracellular parasites.

Arbovirus Studies. With abolishment of NIAID's Laboratory of Tropical Virology, the Arbovirus Unit of our Laboratory became the only facility on the NIH reservation with the professional and technical competency capable of performing studies on arboviruses. The working collection of viral and antibody reagents has expanded considerably. Present reagents representing a number of virus strains for most of the major and minor arbovirus groups are available and being utilized for routine and special studies.

A comparison of several methods for preparing arbovirus hemagglutination (HA) and complement-fixation (CF) antigens demonstrated that adequate HA antigens could be made simply by extraction of suckling mouse brains (SMB) suspensions with genetron and that satisfactory CF antigens could be made from 20X virus-infected cell concentrates, crude and genetron extracted SMB suspensions.

In addition, our laboratory (in collaboration with Biomedical Engineering and Instrumentation Branch, DRS) completed development of a negative pressure isolator modified for work with larger animals. Certain features of this isolator make it more versatile than previously described isolators made from flexible plastic. This isolator allowed us to isolate the virus of simian hemorrhagic fever with absolute safety to other monkeys. Experiments using potential human pathogens could be done with a similar degree of safety.

Hemorrhagic Fever Studies. Previous reports described this Laboratory's isolation of the heretofore unknown virus of simian hemorrhagic fever which was responsible for a devastating epizootic of hemorrhagic fever among colonies of rhesus monkeys at the Primate Quarantine Unit, LAB (Fall, 1964), and at the Sukhumi Institute of Experimental Pathology and Therapy, USSR (July 1964). We have shown that a subsequent epizootic at the National Center for Primate Biology, Davis, California (September 1967) was caused by the same virus. Recent reports suggest that a similar disease occurred in a monkey colony in Great Britain. The significance of knowing more about potential hazardous diseases in monkeys is obvious.

Studies were also performed on 2 members of the Tacaribe group of American hemorrhagic fever viruses. Adult guinea pigs immunized with live Amapari virus did not resist challenge with Junin virus (Argentinian hemorrhagic fever virus) to which they are normally quite susceptible. This was quite unexpected because, as reported earlier, immunization with Tacaribe virus (another member of the same virus group) did protect guinea pigs from subsequent challenge with Junin virus.

Immunobiological Studies. Of major concern in studies of delayed hypersensitivity has been the development of an in vitro test, particularly for man, and the discovery of the factor or factors that confer a passive hypersensitivity state. Previous studies using human RNA species in vitro have shown the transfer of an immunological message to previously nonsensitive human lymphoid cells. Defining the mechanism of activity is paramount for evaluating RNA as a biologic product.

Other studies were performed to evaluate the relative importance of cellular and humoral factors in determining individual responses to revaccination. Following revaccination, volunteers with major skin reactions to vaccinia demonstrated: (1) significant increases in lymphocyte thymidine incorporation compared to pre-immunization levels of reactivity, and (2) the appearance of antigen specific migration-inhibition-factor (MIF).

Studies directed toward the isolation and immunological characterization of cell antigens revealed that antigens with strikingly different immunochemical patterns could be isolated from cell extracts. Additional studies are required to determine the significance of these findings. Histocompatibility antigens and cell-associated antigens obtained in a water soluble state offer many advantages over whole cells in regard to antigenic identification. This work shows promise in such areas as: (1) identification of culture variability and antigenicity; (2) control and standardization of transplantation immunology; (3) selectivity and potency evaluation of antilymphocyte globulin; (4) tumor cell identification and standardization; and (5) a better understanding of autoimmune disorders.

Viral Pathogenesis Studies. A new class of unusual agents found to cause diseases in man (kuru, subacute sclerosing panencephalitis) and animals (scrapie, visna, maedi) show unusual properties, such as long latent incubation period of months to years, protracted disease course, and pathologic changes unlike those of acute viral diseases. Studies with this new class of infectious agents and possible contaminants of biological products will provide DBS with the competency to develop testing and monitoring techniques.

Additional studies were performed on autoimmune reactions related to viral and adjuvant sensitization and on improving techniques for isolation and study of viral agents affecting the central nervous system.

Cell Culture Studies. A program of research and development in connection with production of cell cultures for the Division is actively pursued. Studies involved: (1) standardization of techniques applicable to large-scale production of cell cultures; (2) development of new cell culture systems as possible substrates

for vaccine production and testing; (3) determining the viability of trypsinized tissues at 4C and the use of serum protein fractions in cell culture system; and (4) identification of specific in vitro cell differential antigens.

As a result of these studies, many practical improvements have been introduced into routine cultivation of cells. Increasing emphasis is being given to studying the general biology and immunobiology of cultured cells.

C. CONTRACT ACTIVITIES

There were two contracts which essentially provided services to our Laboratory. One with Melpar, Inc. furnished electron microscope facilities for the study of vaccinia and other viruses (Report #124). This contract was terminated on 21 May 1969, as electron microscope services are now supplied by the Laboratory of Biophysics and Biochemistry, DBS.

The second contract was with Bionetics Research Laboratories, Inc. for the housing and care of rhesus monkeys to be used in this Laboratory's yellow fever and hemorrhagic fever studies. This contract was terminated 31 January 1969.

D. SERVICE AND CONTROL ACTIVITIES

Cell Cultures. The Cell Biology Section continues to spend most of its effort on the purely service function of providing the entire Division with quality cell cultures for research and control uses. Much effort has been made to improve the Section's service functions and to insure adequate cell production. This service results in a considerable saving to the Division and ultimately the taxpayer. All of the DBS continuous cell lines have been replaced with certified, Mycoplasma-free cells and the cell repository is operating at an efficiency which should allow almost immediate return to full scale production in the event of accident or contamination.

Table 1 lists the cell lines currently held in the Laboratory's Cell Biology Section cell repository. Production records of primary and serial cell cultures are summarized in Tables 2 and 3. In addition, 4,968 liters of media were prepared for use in the Cell Production Unit of the Section. Additional time was spent on safety testing of inactivated virus vaccines prepared in cell cultures.

Avian Leukosis Testing. The Avian Leukosis and Immunofluorescence Units are responsible for testing vaccines for potential contamination with viruses of the avian leukosis complex. In addition they routinely check egg derived vaccines for the presence of other avian contaminants (e.g. reticuloendo-

theliosis (RE) virus). During this reporting period a total of 251 RIF and FAB (fluorescent antibody) tests were performed, of which 9 were on 8 live measles vaccines, 2 on lots of experimental rubella vaccines, 6 on 5 rubella vaccines submitted in support of license application, 3 on lots of yellow fever vaccines, and 21 were tests on an experimental measles vaccine. All these tests were negative.

Our Laboratory is also responsible for monitoring the NIH RIF-free chicken flock. There were 210 tests performed on eggs from SPAFAS and the NIH flocks, egg yolks and chick embryo cell cultures.

Infleunza Vaccines. With the onset of this season's influenza epidemic caused by the A2/Hong Kong/68, LVR's Respirovirus Unit and the Division's Laboratory of Control Activities mobilized their forces: (1) to obtain and distribute to the manufacturers a virus strain suitable for vaccine manufacture; (2) to prepare, standardize, and distribute a reference vaccine (which is being regarded as one of the best reference influenza vaccines); and (3) to streamline channels of communication so that vaccine lots could be released, at times, within 24 hours of receipt of a satisfactory protocol from the manufacturers. Because of our successful efforts to make the CCA test simple and reproducible, potency testing of the monovalent vaccine was not the chore it used to be. In addition, reference vaccines of civilian (69C) and military (69M) formulation have been prepared for distribution to the manufacturers for the forthcoming 1969-70 season. The rapidity with which these reference vaccines were prepared and certified by testing attests to the very practical benefit derived from this year's control-related research efforts.

Table 4 summarizes the potency tests performed on influenza vaccine lots submitted during this past year.

Table 1
Report for Calendar Year 1968
REPOSITORY OF EXPERIMENTAL CELLS IN LIQUID NITROGEN

Source (Designation)[a]	Frozen Passage No.
Embryonic bovine kidney	6,10
Embryonic bovine liver	3,8
Embryonic bovine thymus	7,28,31
Embryonic bovine spleen	3,7
Embryonic bovine lung	8,29,33
Embryonic bovine heart	6
Chick Embryo (#251)	1,11,12,14,19
Chick Embryo (#252)	1
Duck Embryo	22
Rabbit Kidney	1,5,8,34
Rabbit Spleen	6
Rabbit Liver	6
Rabbit Lung	4
Rabbit Heart	1
Rabbit Thymus	5
Swine Kidney	4
Opossum Kidney	27,34
Snake Kidney	3
Rhesus Monkey Kidney (BSTC-224)	18,20
Rhesus Monkey Liver	9
Vervet Monkey Brain	5
Vervet Monkey Spinal Cord	4

[a] If designation is other than source, it is shown in parenthesis
[b] Cells available to DBS investigators

Table 2
Report for Calendar Year 1968
NUMBER OF PRIMARY CELL CULTURES ISSUED

Type of Culture	Suspension	Tubes	Bottles		
			2 oz.	MDB*	32 oz.
Kidney					
Monkey, rhesus	400 ml	86,360	2,128	33	158
Monkey, cercopithecus	1,525	145,845	8,140	1,209	168
Rabbit	1,300	31,172	4,810	300	121
Embryo					
Chick	-	220	13,201	-	4
Hamster	-	220	9,183	-	6

*MDB = milk dilution bottles

Table 3
Report for the Calendar Year 1968
NUMBER OF SERIAL CELL CULTURES PREPARED FOR ISSUE AND STOCK BOTTLES MAINTAINED

Type of Culture	Suspension	Tubes	Bottles			Stock Bottles	
			2 oz.	MDB*	32 oz.	32 oz.	Roller
Human							
WI-38	430	16,430	2,060	72	340	574	476
HEp-2	300	4,555	30	56	199	212	
Chang's Liver	300	832	25	-	-	107	
Animal							
MA-104	10,050	24,249	26,572	-	297	2,630	
RK-13	675	46,750	11,614	251	149	1,961	
BS-C-1	1,800	1,950	3,423	2,704	57	2,973	
L	-	6,867	100	-	-	488	
BHK-21	-	3,261	1,579	152	291	706	

*MDB = milk dilution bottles

Table 4
Report for Calendar Year 1968
SUMMARY OF POTENCY TESTS ON INFLUENZA VACCINES

Manufacturer No.	Vaccine Type[a]	Total Lots Submitted (No.)	Lots Tested (No.)	Lots Released (No.)	Lots Rejected or Withdrawn by Mfr.
1	M	14	14	13	1
	CBP	5	5	4	1
2	M	10	10	5	5
	CBP	3	3	3	0
	Mil.	2	2	2	0
3	M	21	21	21	0
	CBP	7	7	7	0
4	M	5	5	5	0
	CBP	9	9	9	0
5	M	7	5	7	0
	CBP	16	14	16	0
6	M	7	7	5	2
	CBP	8	8	8	0
Total	M	64	62	56	8
	CBP	48	46	47	1
	Mil.	2	2	2	0

[a] Vaccine type: M = monovalent A2/Aichi/2/68 vaccine; CBP = civilian bivalent and polyvalent vaccines; Mil. = military polyvalent vaccines.

Serial No. 57
1. Virology & Rickettsiology
2. Office of the Chief
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Office of the Chief

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 4.67
Professional: 0.67
Other: 4.0

Project Description:

Objectives: Administration, planning and coordination of research and control activities of the Laboratory's sections.

The project furnishes supervisory and administrative services to all research projects and biologics control activities in the laboratory as follows: (1) over-all planning of intramural research, collaborative (contract) and control functions and their coordination among and within the several Sections of the Laboratory; (2) supervision over personnel, maintenance, travel and correspondence; (3) supervision over requisitioning and maintenance of equipment and supplies; (4) reviewing, editing and revising scientific and technical reports and manuscripts; (5) preparation of administrative reports, exhibits and other publication materials; (6) participating with other Laboratory Chiefs in staff functions for Office of the Director, including staff committees which ensure smooth operation of the Division in its many types of activity; (7) official liaison and communications with the manufacturers of viral vaccines and other biologics; (8) consultation services to individuals, academic institutions and government agencies, including liaison activities with other branches of the Service, foreign governments and international agencies; (9) maintenance of reference library and reprint files.

Honors and Awards:

1. Fellow, American Academy of Pediatrics (elected 16 October 1968).

2. Consultant, Department of Medicine, Children's Hospital of the District of Columbia.

3. Invited participant, Workshop on Virus Diseases Which Impede Colonization of Nonhuman Primates, National Center for Primate Biology, University of California, Davis, California, 22-24 May 1968 Simian Hemorrhagic Fever); moderator of session on Infectious Hepatitis.

4. Invited speaker, 25th Anniversary Reunion of NAMRU-2, Washingtonian Motel, Gaithersburg, Maryland, 26 October 1968 (The Need for Self-Discipline).

5. Invited consultant in virology, Delta Regional Primate Center, Covington, Louisiana, 15-16 April 1969.

6. Invited lecturer, U.S. Naval Hospital, National Naval Medical Center, Obstetrics and Gynecology Service, Bethesda, Maryland, 19 April 1969 (Virus Infections and the Use of Vaccines during Pregnancy).

7. Invited participant, International Symposium dealing with A Human Disease Caused by an Infectious Agent (Marburg Virus) Acquired from African Vervet Monkeys, University of Marburg, Marburg/Lahn, Western Germany, 26-27 June 1969.

Serial No. 139
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on autoimmune reactions related to viral and adjuvant sensitization

Previous Serial No.: Same

Principal Investigator: Paul Albrecht, M.D.

Other Investigators: Alexis Shelokov, M.D.
Ronald Clark, Ph.D.
Ruth Kirschstein, M.D.

Cooperating Units: Laboratory of Pathology, DBS

Man Years:
Total: 0.5
Professional: 0.2
Other: 0.3

Project Description:

Objectives: (1) Select a suitable animal model (preferably mouse) for measuring of humoral and cellular immunity to viruses under consideration, e.g., herpes simplex, measles, arboviruses and rabies; (2) evaluate the effect of repeated injection of adjuvant alone, adjuvant plus viral antigens and accompanying tissue components, and virus alone on tissue susceptibility and tissue vulnerability of the sensitized host.

Methods Employed: Animals: Besides two multiple-branched colonies (CFW and the NIH General Purpose strain derived from white Swiss mice) one each representative of the A, AL, AV-R, BALB/C, DBA/2A mouse strains, two representatives of the C3H and four of the C57 and C58 lines were used. Further nine more recently developed inbred strains were included, mainly the New Zealand black, New Zealand white and P/N strains. These were examined against the background of strain B.S.V.S. considered the only one susceptible to allergic encepahlitis (one line from the Rockefeller Institute, New York, one line from the Wistar Institute, Philadelphia).

The encephalitogen was used in complete Freund adjuvant using the mycobacteria strain H_{37} RV. In selected instances guinea pig spinal cord, basic protein or human brain were used. To increase exposure of the CNS to sensitized lymphocytes transfer of spleen cells from immunized mice into isogenic X-irradiated (800 r) host animals was performed.

Major Findings: None of the mouse lines inoculated showed unequivocal symptoms of encephalitis and none died with confirmed lesions of encephalitis. Histological examination showed sporadic mild inflammatory lesions in strains C 3HfB/HeN, A/HeN, AL/N and STR/N present only in a few of the inoculated animals. The slight intensity and extent of lesions would make their separation from spontaneous lesions difficult. The conclusion has been reached that none of the 23 strains of mice tested, including strain B.S.V.S. are capable of developing allergic reactions of the CNS comparable to guinea pigs or rats (Lewis strain) so as to be useful in test on neuroallergenic properties of biological preparations. Further tests in mice were abandoned.

Significance to Bio-Medical Research and the Program of DBS: It was considered of advantage to have available a strain of the most convenient laboratory animal, the mouse, for tests on autoimmune mechanisms which become involved by the action of biologic preparations.

Proposed Course of the Project: The objectives as given above will be pursued in tests on rats.

Honors and Awards: None

Publications: None

Serial No. 140
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on agents of chronic and degenerative diseases

Previous Serial Number: Same

Principal Investigator: Paul Albrecht, M.D.

Other Investigators: W. D. Hann, Ph.D.
J. Oppenheim, M.D.

Cooperating Units: Bowling Green State University, Bowling Green, Ohio
Laboratory of Biochemistry, NIDR

Man Years:
Total: 1.2
Professional: 0.5
Other: 0.7

Project Description:

Objectives: A new class of agents has been defined, causing disease in man (kuru, spongiform encephalopathy, Jakob-Krenzfeld disease) and animals (scrapie, visna, maedi, NZB mouse agent) which show unusual properties, e.g. latency, long incubation period--months to years; protracted course of serious disease, pathological changes unlike those of acute viral disease.

Some of the agents are unusually resistant to heat, formalin, radiation and pH. They seem to be ubiquitous with the manifestations of disease dependent on the genetic constitution of the host and circumstances of transmission.

As a rule, the cultivation and identification of these agents poses a formidable problem. To monitor confidently for the presence of "slow virus" agents in biological materials, new assay systems and a better understanding of the biology of these agents are necessary.

Methods Employed: Early detection of scrapie infection. During early stages of intracerebral multiplication of scrapie a heterogenic brain specific antigen is produced in astrocytes undergoing a marked hypertrophy. The demonstration of this antigen by fluorescent antibodies in mice used for infectivity assays cut by half the time of 7-8 months usually necessary for LD_{50} endpoints.

Major Findings: Interference of scrapie with conventional viruses. The finding that scrapie agent is widely distributed in the lymphatic system before invading the nervous system led to investigations on possible interference with viruses displaying similar pathogenesis. It was hoped that besides general information an indicator system could be found for the extraneural phase of scrapie infection.

Powassan, pseudorabies, vesicular stomatitis, measles, Rauscher and Friend leukemia viruses were used as challenge agents in mice infected by the intraperitoneal (IP) and intracerebral (IC) routes. So far consistent interference has been found between scrapie and Powassan virus 3-6 weeks after infection with a peak at 4 weeks. Titers of Powassan virus were up to 100-fold lower in scrapie as compared to control mice. Interferon production in scrapie mice was not different from control animals. Preliminary studies on antibody response to viral and non-infectious antigens in scrapie mice do not show marked differences from the response in normal mice.

Protective immunity in scrapie infection was studied in several groups of animals. Mice immunized with three doses of heat inactivated scrapie in complete Freund adjuvant, mice immunized with active scrapie, control groups which received inactivated or noninactivated brain or placebo. All groups were challenged 4 weeks later by the IC or IP route. Contrary to expectation mice preimmunized with active and inactive scrapie material had shortened incubation periods compared to nonimmunized mice. Under given conditions, no humoral or cellular protection could be induced against scrapie. The shorter incubation period in immunized animals was probably due to the nocuous effect of brain tissue in the immunizing inoculum.

Significance to Bio-Medical Research and the Program of DBS: Dealing with a new class of infectious agents and possible contaminants of biological products, the studies are intended to give competence to DBS workers in this field and join in developing monitoring techniques and measures.

Proposed Course of the Project: The project will continue as outlined.

Honors and Awards: None

Publications: None

Serial No. 173
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Improved techniques for isolation and study of viral agents affecting the nervous system

Previous Serial Number: None

Principal Investigator: Paul Albrecht, M.D.

Other Investigators: Roger Snyder, M.D.
Heinz Schumacher, M.D.

Cooperating Units: Department of Biochemistry, NINDS

Man Years:
Total: 1.5
Professional: 0.8
Other: 0.7

Project Description:

Objectives: Inconsistencies or failures to isolate a causative agent in acute and chronic diseases of the CNS constitute a major handicap in evaluating the pathogenesis of infection. Inhibitors and/or specific antibodies suppressing the assumed low amount of viral agent are incriminated. The difficulty to prove the measles etiology of subacute sclerosing panencephalitis is one of the more recent examples.

Methods Employed: Neurotropic measles virus growing in hamster, rat, and mouse brain was used to infect the respective species. Virus was isolated by intracerebral inoculation into the respective species, into BS-C-1, MA-104, GMK, RMK and BHK cells.

Two approaches were considered to eliminate the effect of brain inhibitors and antibodies: (a) growing nervous tissue into monolayers and subsequent inoculation into conventional tissue culture, (b) dissociation of nervous tissue by chemical or enzymatic means.

The success of the first approach is based on availability of viable brain specimens processed immediately after biopsy. As dissociation of brain tissue can be practiced on post mortem and frozen samples, preference to this approach was given.

Major Findings: Inhibition of virus by 10% brain homogenates. Suckling hamster brain infected with neurotropic measles virus will contain about 10^4 $TCID_{50}$ of virus. However, when a 10% suspension or homogenate of brain tissue are applied to the cell culture, only 1-2% of infectivity will be recovered. This inhibitory effect will also appear when cultures are exposed to non-infected brain tissue concommitantly, but not previous to the virus. Brains from different animal species have this effect. As inhibition of virus is reversible, ways of "neutralizing" the inhibitors are considered.

Dissociation of brain tissue. Tetraphenylboron (TPB), a potent chelating agent of potassium was recently introduced for dissociation of parenchymal tissues. In experiments with measles-infected brain we employed a .005 M solution of TPB for different times at +4 C. A morphologically well preserved suspension of nerve and glial cells was obtained. In all instances toxicity of TPB for the cells impaired considerably the transfer of measles virus to cell cultures.

Dissociation of brain tissue employing trypsin and subsequent purification on a ficol gradient proved an efficient way as compared to isolation from crude whole brain homogenates. Purification and concentration used with TPB had to be modified. Examination of optimal conditions to achieve separation of cell debris and lipoid substances is in progress.

Sensitivity of cell cultures for isolation of neurotropic measles virus. Using different culture systems and isolation procedures the most sensitive technique involved incubation of the inoculum with suspended BS-C-1 cells followed 2-3 days later by transfer into suspended MA-104 cells. Two to three 48 hour subpassages at times were necessary for a visible CPE to develop. Through 12 passages in MA-104 cells the virus remained neurotropic and could be transferred only with intact cells. Further work is aimed at creating conditions for a complete reproduction cycle in tissue cultures and determination of the basic properties (markers) of a neurotropic measles virus.

Significance to Bio-Medical Research and the Program of DBS: To find optimal procedures for isolation of viral agents from the central nervous system is desirable especially with agents attenuated strains of which are used in live vaccine preparations.

Proposed Course of the Project: The project will continue at the present level.

Honors and Awards: None

Publications:

Abels, B. R., Gajdusek, D. C., Gibbs, C. J., Jr., Albrecht, P., and Rogers, N.: Attempts to transmit subacute sclerosing panencephalitis and isolate a measles related agent, with a study of the immune response in patients and experimental animals. Neurology, 18: 30-51, 1968.

Serial No. 173

Albrecht, P.: Pathogenesis of neurotropic arbovirus infections. Current Topics in Microbiology and Immunology, 43: 44-91, 1968.

Serial No. 75
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on arthropod-borne viruses (arboviruses)

Previous Serial Number: Same

Principal Investigators: Nicola M. Tauraso, M.D.
Frank A. Pedreira, M.D.

Other Investigators: George F. Norris
Murrel L. Myers

Cooperating Units: Biomedical Engineering and Instrumentation Branch, DRS

Man Years:
Total: 0.57
Professional: 0.28
Other: 0.29

Project Description:

Objectives: (1) To maintain a laboratory facility with competency in the techniques of animal work, cell culture and serology for the study of arboviruses; (2) to maintain a reference collection of arboviruses, including those of importance as vaccine candidates; and (3) to conduct studies on select arboviruses, especially those of importance as potential vaccine candidates.

Major Findings: The arbovirus facility now has the professional and technical competency capable of performing studies on the arboviruses, utilizing the techniques of animal inoculation, cell culture and serology.

The working collection of viral and antibody reagents has expanded considerably. Present reagents representing a number of virus strains for most of the major and minor arbovirus groups are available and are being utilized for routine and special studies. During this year studies were continued with emphasis on simian hemorrhagic fever, American hemorrhagic fever and yellow fever vaccine viruses (reported separately under project Nos. 118 and 121 respectively).

A comparison was made of several methods for preparing arbovirus hemagglutination (HA) and complement-fixation (CF) antigens. Our results demonstrated that adequate arbovirus HA antigens could be made simply by extraction of suckling mouse brain (SMB) suspensions with genetron and that satisfactory CF antigens could be made from 20X virus-infected cell concentrates, crude and genetron extracted SMB suspensions.

In addition to these studies, our laboratory completed development of a negative pressure isolator modified for work with larger animals. Certain features of this isolator make it more versatile than previously described isolators made from flexible plastic. Because it is constructed chiefly from stainless steel it is easy to clean and animals are unable to eat their way out. Although many different size cages can be placed within the basic isolator allowing work with animals as small as mice, we feel the greatest advantage is with work requiring larger animals such as the monkey. Using this isolator we have been able to perform studies which otherwise could not have been done. Our studies allowed us to isolate the virus of simian hemorrhagic fever with absolute safety to other monkeys. Experiments using potential human pathogens could be done with a similar degree of safety.

Significance to Bio-Medical Research and the Program of DBS: Many of the continental states, as well as Hawaii, Alaska and Puerto Rico, possess the ecological prerequisites of potential vectors and reservoirs necessary for initiation of arbovirus epidemics. Vaccines against arboviruses of importance to the world--the U.S. personnel and travelers abroad--are being developed. Some may soon come within the purview of the Division of Biologics Standards. Further knowledge of the biochemical and biophysical properties is needed in order to appreciate more the unique features of arboviruses and before these viruses can be handled as biologic products.

Proposed Course of the Project: Research on selected arboviruses will continue.

Honors and Awards: None

Publications:

Pedreira, F. A., Tauraso, N. M., Klutch, M. J., and Shelokov, A.: A comparison of several methods for preparing arbovirus hemagglutinating and complement fixing antigens. Amer. J. Trop. Med. & Hyg. (in press) 1969.

Tauraso, N. M., Norris, G. F., Sorg, T. J., Cook, R. O., Myers, M. L., and Trimmer, R.: A negative-pressure isolator for work with hazardous infectious agents in monkeys. Amer. J. Trop. Med. & Hyg. (in press) 1969.

Serial No. 118
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on hemorrhagic fever viruses

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: Carlos D. Espana, M.D.
Alexis Shelokov, M.D.

Cooperating Units: National Center for Primate Biology, Davis, California

Man Years:
Total: 0.54
Professional: 0.21
Other: 0.33

Project Description:

Objectives: A. (1) To maintain a facility for working with the important viruses causing hemorrhagic fever in the western hemisphere with emphasis on the immunological vaccine aspects; (2) to maintain practical cell culture and animal host systems and serological tools for working with this group of viruses; (3) to study the immunological relationships of these viruses using in vitro serological tests and in vivo animal protection tests.

B. (1) To study the pathophysiology of hemorrhagic fever using monkeys infected with simian hemorrhagic fever (SHF) virus as a model; (2) to attempt to isolate a virus from specimens obtained from monkeys which died of hemorrhagic fever at the National Center for Primate Biology, University of California at Davis in September, 1967 and to determine the relationship of that disease with the hemorrhagic fever which occurred at the Primate Quarantine Unit, LAB, NIH in the Fall of 1964 and at the Sukhumi Institute of Experimental Pathology and Therapy, USSR, in July, 1964.

Methods Employed: A. Standard virologic and serologic methods were employed in these studies. B. Utilizing the broad resources available to the Arbovirus Unit, LVR, an attempt was made to establish the etiology of a simian hemorrhagic fever occurring at the National Center for Primate Biology at Davis, California.

Major Findings: A. Studies were performed on 2 members of the Tacaribe group of American hemorrhagic fever viruses. Adult guinea pigs immunized

with live Amapari virus (not known to be pathogenic for the adult guinea pig) did not resist challenge with Junin virus (Argentinian hemorrhagic fever virus) to which they are normally quite susceptible. This was quite unexpected because, as reported in an earlier annual report, immunization with Tacaribe virus (another member of the same virus group) did protect guinea pigs from subsequent challenge with Junin virus.

B. Serum samples were obtained from monkeys involved in the Davis epizootic of simian hemorrhagic fever. Sera were selected and inoculated intramuscularly into rhesus monkeys housed in a specially constructed negative pressure isolator. The inoculated monkeys developed a disease indistinguishable from that seen during the NIH epizootic. We were unable to isolate a virus from the sera of these monkeys, even after many repeated attempts. However, serum obtained from monkeys dying late in the course of the disease contained antibodies to the viruses which had been isolated several years previously from both the NIH and Sukhumi epizootics. Clinically, the disease involved in the three epizootics is indistinguishable. We have shown previously that the NIH and Sukhumi virus strains are serologically similar and the one-way serologic cross between antibody to the Davis virus strain and the NIH and Sukhumi strains demonstrates that the Davis epizootic was caused by a similar or closely-related virus.

Significance to Bio-Medical Research and the Program of DBS: A. Concerning the studies on the American hemorrhagic fever viruses, there is no doubt that viral hemorrhagic fevers have become a major public health concern in the Americas. So far attempts to provide a safe and adequate vaccine against the Argentinian disease have been unsuccessful. During the most recent epidemics of Bolivian hemorrhagic fever, much consideration was given to the possibility of immunization as an eventual control measure. It would be in keeping with the mission of this Division to develop and maintain the competency able to cope with the problems of evaluating the potential vaccine candidates.

B. Since primates are needed by the Division in carrying out the required safety tests on some vaccines, it is only logical that the arbovirus facility of LVR maintain a competency able to cope with certain aspects of endogenous viral diseases of monkeys.

During the years of 1964 to 1967 there have been three known outbreaks of simian hemorrhagic fever. Recent reports suggest that a similar disease occurred in a monkey colony in Great Britain. The significance of knowing more about potential hazardous diseases of monkeys is obvious.

Proposed Course of the Project: Studies on the American hemorrhagic fever and simian hemorrhagic fever viruses will be continued.

Honors and Awards:

Invited participant (Dr. Shelokov)--National Center for Primate Biology Workshop on Virus Diseases which Impede Colonization of Nonhuman Primates, Davis,

California, May 22-24, 1968 (Epizootiology of Simian Hemorrhagic Fever); moderator of session on Herpesviruses.

Publications:

Tauraso, N. M., Shelokov, A., Allen, A. M., Palmer, A. E., and Aulisio, C. G.: Simian hemorrhagic fever: Isolation and identification of the virus causing an epizootic in Sukhumi, USSR, Nature, 218: 876-877, 1968.

Serial No. 121
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on Yellow Fever Vaccine

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: William G. Jahnes
Ruth L. Kirschstein, M.D.
Edward B. Seligmann, Jr., Ph.D.

Cooperating Units: Laboratory of Control Activities, DBS
Laboratory of Pathology, DBS

Man Years:
Total: 1.83
Professional: 0.33
Other: 1.50

Project Description:

Objectives: (1) To purify the current Yellow Fever Vaccine by removing the contaminating avian leukosis virus; (2) to evaluate the neurovirulence of the new vaccine candidate in monkeys; (3) to study the immunogenicity of the purified vaccine candidate in monkeys and man; and (4) to attempt to select by plaque-purification a strain with less neurovirulence than the current vaccine strain.

Methods Employed: Utilizing the broad resources available to the Arbovirus, Immunoserology, and Avian Leukosis Units, LVR an attempt is being made to investigate the various aspects of this project by common laboratory techniques.

Major Findings: The DBS yellow fever project was established in April 1966 as a coordinated research program involving several laboratories in the Division.

The first objective has been achieved. An avian leukosis virus (ALV)-free primary yellow fever vaccine seed has been developed by ridding the 17D virus vaccine of its ALV contaminant. By differential filtration through Millipore membrane filters of

different pore sizes, yellow fever virus was separated from its contaminant by physical means. The ALV could not be detected in the new primary and secondary vaccine seeds by RIF, COFAL, and FAB tests.

The second objective has also been accomplished. ALV-free candidate primary and secondary seed lots were indistinguishable from corresponding ALV-contaminated lots with respect to (1) potency as measured by titration in newborn and weanling mice and in the MA-104 plaque system, (2) degree of viscerotropism as measured by viremia in monkeys, (3) neurotropism as determined by the monkey neurovirulence test, and (4) potency as determined by antibody response in monkeys inoculated by the intracerebral route.

The third objective has almost been completed. A total of 265 rhesus monkeys (Macaca mulatta) were inoculated, 129 with ALV-contaminated and 136 with ALV-free YF vaccine. Table I summarizes the results of hemagglutination-inhibition and plaque neutralization tests. Volunteer studies were conducted at Lewisburg Federal Penitentiary. ALV-contaminated YF vaccine was given to 89 and ALV-free vaccine administered to 91 inmates. These vaccines were indistinguishable with respect to reactions and to antibody response (Table I).

As part of the Yellow Fever Vaccine studies, we have developed a convenient plaque neutralization (PN) test using the MA-104 embryonic rhesus monkey kidney cell line and a single agar-overlay procedure. When compared to mouse neutralization (MN) tests using suckling and weanling mice inoculated by the intracranial route, the PN test was: the most sensitive for measuring neutralizing antibody, less variable, less costly, and quicker (5-6 days vs 21 days for the PN and MN tests respectively) in achieving results.

Significance to Bio-Medical Research and the Program of DBS: A reevaluation of old vaccines using more up-to-date methods is one of the responsibilities of the Division. The development of a Yellow Fever Vaccine free of contaminating avian leukosis virus is obviously advantageous to humans receiving this vaccine.

Proposed Course of the Project: These studies are being continued.

Honors and Awards: None

Publications:

Tauraso, N. M., Spector, S., Jahnes, W. G., and Shelokov, A.: Yellow fever vaccine. I. Development of a vaccine seed free from contaminating avian leukosis viruses. Proc. Soc. Exp. Biol. & Med. 127: 1116-1120, 1968.

Tauraso, N. M., Spector, S. L., Kirschstein, R. L., Seligmann, E. B., and Farber, J. F.: Yellow fever vaccine. II. Antigenicity and neurovirulence of a vaccine seed free from avian leukosis virus. Appl. Microbiol. (in press) 1969.

Spector, S. L., and Tauraso, N. M.: Yellow fever virus. I. Development and evaluation of a plaque neutralization test. Appl. Microbiol. 16: 1770-1775, 1968.

Serial No. 121

Results of Serological Tests Performed on Monkeys and Humans Inoculated with Avian Leukosis Virus Contaminated and -Free Yellow Fever (YF) Vaccine

Host	Group	ALV Status of Vaccines: Contaminated				ALV Status of Vaccines: Free			
		No. in Group	HI[a] %	PNT[b] LNI[c]	PNT[b] %[d]	No. in Group	HI %	PNT LNI	PNT %
Rhesus monkeys	1	42(38)[g]	76(76)[h]	2.3[e]	94	40(36)[g]	73(78)[h]	2.5	97
	2	17(17)	77(77)	ND[f]		25(25)	72(72)	ND	
	3	49(47)	78(77)	2.1	100	48(48)	46(46)	1.4	95
	4	21(20)	91(95)	2.4	100	23(23)	91(91)	2.5	100
	Total	129(122)				136(132)			
	average		81(81)	2.3	98		71(72)	2.1	97
Humans	1	89(65)	62(71)	2.2	99	91(68)	69(78)	2.2	98

[a] Percent of individuals developing a 4-fold or greater rise in HI antibody titer.
[b] PNT = plaque neutralization test.
[c] LNI = log neutralization index.
[d] Percent of individuals having an LNI rise of ≥ 0.7
[e] Average value
[f] Not done yet
[g] No. having no YF HI Ab in preinoculation serum
[h] Percent of individuals not having YF HI Ab in preinoculation serum who developed 4-fold or greater rise in HI Ab.

Serial No. 122
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on respiratory viruses.
I. Influenza virus vaccines.

Previous Serial Number: Same and 123

Principal Investigators: Nicola M. Tauraso, M.D.
Frank A. Pedreira, M.D.

Other Investigators: Jose G. Canchola, M.D.

Cooperating Units: Laboratory of Control Activities, DBS

Man Years:
Total: 8.33
Professional: 2.08
Other: 6.25

Project Description:

Objectives: (1) To maintain a competent Respirovirus Unit for the study of influenza viruses and other viral agents etiologically associated with respiratory tract infections of man; (2) to maintain a reference collection of infectious viral antigens and antibody reagents of influenza viruses and other respiroviruses including those of potential importance as vaccine constituents; (3) to review and analyze manufacturers' protocols and to perform immunogenic potency tests on current influenza virus vaccines; (4) to improve serologic techniques for immunogenic potency testing of influenza virus vaccines; (5) to compare reproducibility of serologic tests for immunogenic potency of current influenza virus vaccines; and (6) to conduct immunogenic studies with influenza viruses of epidemiologic importance in the United States.

Methods Employed: Standard virologic and serologic methods were employed in these studies.

Major Findings: Heretofore, the CCA (chicken cell agglutination) test was considered unreliable as a measure of the antigen content of influenza vaccines because the test was not standardized from laboratory to laboratory. During this past

year, great strides have been made to make the CCA test reproducible not only within a laboratory but also between different laboratories. We discovered that it was important to pay close attention to define such things as 1) position of the lamp within the Klett colorimeter, 2) position of the specimen tube at time of reading, 3) length and width of specimen tubes, 4) buffer to be used for diluting vaccines and cockerel red blood cells, etc. Manufacturers were instructed as to such details, and, probably for the first time since its development, the CCA test could be used reliably to measure the antigen content of influenza vaccines. This was especially helpful to us in carrying out our control mission during this season's epidemic caused by the Hong Kong A2 variant.

During this 1968-69 influenza vaccine season we were able to test every lot submitted by the manufacturers. Of 114 lots submitted 106 were released as satisfactory with respect to potency. This volume of testing could never have been achieved before.

DBS was able to make some of its vaccine (ordinarily used for the purposes of control) available to competent investigators early enough so that major volunteer studies were initiated. We have been directly involved in two different studies which were completed prior to the major epidemic wave. Serologic tests are currently in the process of completion and analysis.

In addition, we have almost completed an extensive study to evaluate the relative merits of the egg, mouse and tissue culture neutralization as an immunologic test for measuring potency of influenza vaccines. We are currently analyzing these results by computer.

Reference vaccines of civilian (69C) and military (69M) formulation have been prepared for distribution to the manufacturers for the forthcoming 1969-70 season. The rapidity with which these references were prepared and certified by testing attests to the very practical benefit derived from this year's efforts.

Significance to Bio-Medical Research and the Program of DBS: DBS is responsible for the control and potency testing of current influenza virus vaccines. Sustained and comprehensive scientific research, as performed by the Respirovirus Unit, is essential for the improvement and development of antigenically competent influenza virus vaccines.

Proposed Course of the Project: Studies on influenza viruses and influenza vaccines will continue.

Honors and Awards: None

Publications:

Lee, A. M., and Tauraso, N. M.: A method for the formulation of influenza virus vaccine using numerical taxonomy. Bull. Wld. Hlth. Org. 39: 261-270, 1968.

Pedreira, F. A., Tauraso, N. M., Palmer, A. E., Shelokov, A., and Kirschstein, R. L.: Attempts to infect rhesus monkeys with human Type 4 adenovirus. Proc. Soc. Exp. Biol. and Med. 129: 472-477, 1968.

Serial No. 141
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on endogenous viruses contaminating avian eggs
I. Duck hepatitis virus

Previous Serial Number: Same

Principal Investigator: Nicola M. Tauraso, M.D.

Other Investigators: Calvin G. Aulisio, Ph.D.

Cooperating Units: None

Man Years:
Total: 0.28
Professional: 0.28
Other: 0

Project Description:

Objectives: (1) To study systematically the properties of viruses known to contaminate eggs of avian species; and (2) to search for unknown agents endogenous to avian eggs and to develop tests for detecting these newer virus contaminants.

Major Findings: We were unable to develop an immunofluorescence test for the detection of duck hepatitis virus antibody. Re-evaluation of priorities resulted in our shifting emphasis away from duck hepatitis virus to other potential contaminants of duck eggs.

Significance to Bio-Medical Research and the Program of DBS: When a new host is being considered for propagation of virus to be used for vaccines, it is imperative that knowledge be obtained concerning the endogenous agents of the new host. Although much is known concerning the virus contamination of chicken embryos, little is known about the agents which might contaminate duck embryos. Now that a live virus vaccine (i.e., rubella) produced in the duck embryo has been developed and is being considered for general use, a constant search for and knowledge of potential endogenous contaminating agents would ensure continued safety of the vaccine.

Proposed Course of the Project: Our studies on duck hepatitis virus have essentially been completed. Additional studies on other endogenous viruses will be performed and reported as part of other reports.

Honors and Awards: None

Publications:

Tauraso, N. M., Coghill, G. E., and Klutch, M. J.: Properties of the attenuated vaccine strain of duck hepatitis virus. Avian Diseases (in press) 1969.

Serial No.117
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Avian leukosis viruses in relation to human vaccines

Previous Serial Number: Same

Principal Investigators: William G. Jahnes
Calvin G. Aulisio, Ph.D.

Other Investigators: Nicola M. Tauraso, M.D.
Freddie Mitchell, B.S.
Henry C. Orr, Ph.D.

Cooperating Units: Laboratory Aids Branch, DRS

Man Years:
Total: 1.81
Professional: 1.5
Other: .31

Project Description:

Objectives: (1) To test live and inactivated vaccines prepared from chick embryo tissue or chick embryo cells in tissue culture for the presence of Resistance-Inducing-Factor (RIF); (2) to develop new and more sensitive procedures for detecting viruses of the avian leukosis complex; (3) to compare the relative effectiveness of the RIF, COFAL (complement-fixation for avian leukosis) and fluorescent antibody (FAB) techniques for the detection and assay of several members of the avian leukosis virus complex; (4) to assist in the development of RIF-free vaccines prepared in chick embryos or chick embryo cell cultures.

Methods Employed: Standard and modified cell culture techniques, complement-fixation, and FAB tests are employed.

The study outlined as objective No. 2 has been broadened to include the use of many different primary and continuous cell culture systems to screen for susceptibility to Rous sarcoma virus (RSV) and other viruses of the avian leukosis complex.

Objective No. 3 is a cooperative effort of three groups of investigators within LVR. The Avian Leukosis Unit performs the RIF test according to the standard DBS procedure used in the testing of vaccines; the Immunoserology Unit performs the COFAL test; the Immunofluorescence Unit performs the fluorescent antibody test. Antibody and antigen reagents are prepared and standardized for use in these studies; other techniques are utilized as required, such as cell culture, animal and egg inoculation with viruses, and tumor transplantation. These studies have also been expanded to include an evaluation of the NP (Non-virus producing) test to detect viruses of the avian leukosis complex.

Major Findings: During this reporting period a total of 251 RIF tests were performed. Of these 9 were tests on 8 live measles vaccines which were RIF and FAB test negative; 2 were tests on experimental rubella vaccines, and 6 were tests on 5 rubella vaccines submitted in support of license application, all were found RIF and FAB test negative; 3 were tests on RIF-free yellow fever vaccines; 21 were tests on an experimental measles vaccine being developed commercially; 210 were miscellaneous tests on eggs from SPAFAS and the NIH flock, egg yolks, and CETC. Also included in the latter category were special tests on manufacturer's media and cell cultures, positive and negative controls and virus titrations.

The NP test is still undergoing evaluation as to its adaptation for use in screening vaccines but does not appear promising at this time.

A major portion of our screening effort was expended in the successful establishment of a second RIF-free flock at the NIH Farm at Poolesville. This flock is to supplement and also serve as a back-up flock to the NIH flock in Bldg. 14G.

A program for the study of Marek's disease has been established with procurement of isolators, isolation laboratory space, and three of the several candidate strains of the agent. (See Project Report No. 163).

Significance to Bio-Medical Research and the Program of DBS: At present, the RIF test is routinely performed on vaccines for detecting viruses of the avian leukosis complex. The FAB test provides a rapid double check on vaccine testing and is a quick, easy method of continuous monitoring of RIF-free flocks. Because RIF-free chick embryo fibroblasts are required in the safety testing of vaccines, a RIF-free chicken flock must be maintained at NIH. The monitoring of this flock can be rapidly and easily achieved by using techniques to test eggs for maternal antibody

by the indirect FAB technique. The development of more sensitive and more reliable procedures is of utmost importance to the Division's mission.

Proposed Course of the Project: Work will continue on improving the present RIF, FAB, and COFAL tests and on developing simpler and more rapid procedures for the detection of the avian leukosis viruses. Studies on Marek's disease, a known important cause of avian leukosis, has been initiated with the hope of developing a satisfactory test system.

Studies are continuing on the preparation of a RIF-free tissue culture yellow fever vaccine and its storage viability and evaluation of stabilizers.

Honors and Awards:

1. Invited participant (Mr. Jahnes) - The NP Test for the Avian Leukosis Viruses. United States Department of Agriculture, Poultry Research Branch, Animal Husbandry Research Division, Agriculture Research Service, Regional Poultry Research Laboratory, East Lansing, Michigan.

2. Invited participant (Dr. Aulisio) - Same

Publications:

Tauraso, Nicola M., Spector, Sheldon L., Jahnes, William G., and Shelokov, Alexis: Yellow Fever Vaccine. I. Development of a Vaccine Seed Free from Contaminating Avian Leukosis Viruses. Proc. Soc. Exptl. Biol. & Med. 127:1116-1120.

Serial No. 125
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Egg yolk antibody as a tool to study avian microbial infections potentially contaminating human vaccines

Previous Serial Number: Same

Principal Investigator: Calvin G. Aulisio, Ph.D.

Other Investigators: Alexis Shelokov, M.D.
Nicola M. Tauraso, M.D.

Cooperating Units: U.S. Department of Agriculture, Washington, D.C.

Man Years:

Total:	61
Professional:	3
Other:	31

Project Description:

Objectives: To study the nationwide prevalence of avian microbial infections present in chicken and duck eggs which may potentially contaminate human vaccines.

Methods Employed: Indirect immunofluorescence employing egg yolk and serum as sources of antibody.

Major Findings: We demonstrated the usefulness of substituting egg yolk antibody for serum antibody in the immunofluorescence test to study agents of all microbial groups (viruses, rickettsiae, bedsoniae, mycoplasmas, and bacteria). Eggs from 41 of 93 American chicken flocks had antibody to reticuloendotheliosis (RE) viral antigen. Although, RE virus causes a clinical syndrome similar to avian leukosis viruses, we were unable to demonstrate the presence of RE virus as a contaminant in human vaccines. A total of 54 vaccines (10 mumps, 17 measles, 13 rubella, and 14 yellow fever) were examined. Our findings suggest that RE virus is not an important contaminant in human vaccines prepared from RIF-free eggs. We found young chicks and young ducks to be susceptible to experimental infection with group C avian leukosis viruses. The group C avian leukosis viruses may be another potentially important contaminant in chicken and duck eggs.

The capability of the immunofluorescence test to detect these different avian leukosis subgroups makes it an important adjunct to the RIF test in the safety testing of human viral vaccines of egg origin (see Serial No. 117).

Significance to Bio-Medical Research and the Program of DBS: Some licensed and experimental viral and rickettsial vaccines are prepared in chicken and duck eggs. We now have a simple means to study the occurrence of various avian microbial infections in fowl. This may have broad biological and veterinary significance, besides providing the vaccine manufacturers and commercial breeders with a rapid method to screen their flocks for avian leukosis virus antibodies.

Proposed Course of Project: To continue with the improvement and development of immunofluorescence techniques for the detection of adventitious agents in human viral vaccines of egg origin.

Honors and Awards: None

Publications:

Aulisio, Calvin G. and Alexis Shelokov: Prevalence of reticuloendotheliosis in chickens: Immunofluorescence studies. Proc. Soc. Exp. Biol. & Med. 130: 178-181, 1969.

Serial No. 163
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies to elucidate the cause and/or etiological agent causing Marek's disease in chickens

Previous Serial Number:

Principal Investigators: Calvin G. Aulisio, Ph.D.
William G. Jahnes
Other Investigators: Nicola M. Tauraso, M.D.

Cooperating Units: None

Man Years:

Total:	91
Professional:	6
Other:	31

Project Description:

Objectives: (1) To find a satisfactory explanation for the cause of Marek's disease, (2) to determine whether Marek's disease can be transmitted via the egg, the extent and significance of potential contamination, and (3) to develop satisfactory methods for testing potential contamination in chicken embryos to be used for vaccine production.

Methods Employed: The inoculation of cell cultures, embryonated eggs and newborn chicks with candidate strains of Marek's disease and non-related microbial agents. The use of conventional tests, such as, indirect immunofluorescence, complement-fixation, neutralization, gel-diffusion, and interference.

Major Findings: This is a new project.

Significance to Bio-Medical Research and the Program of DBS: Marek's disease at the present time is the major cause of death and/or condemnation of poultry in the U.S. and abroad. It is of the utmost importance to the control of activities of the Division that diagnostic tests be devised for screening vaccines of avian origin.

Proposed Course of the Project: These studies have recently been initiated and will continue as outlined.

Honors and Awards: None

Publications: None

Serial No. 164
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on endogeneous viruses contaminating avian eggs. II. Group specific antibody of avian leukosis viruses in experimentally infected pigeons

Previous Serial Number: None

Principal Investigators: Calvin G. Aulisio, Ph.D.
William G. Jahnes

Other Investigator: Nicola M. Tauraso, M.D.

Cooperating Units: None

Man Years:

Total:	91
Professional:	6
Other:	31

Project Description:

Objectives: To extend previous studies in pigeons experimentally infected with Rous sarcoma viruses and to determine the feasibility of using pigeon antibody to detect avian leukosis viruses in human vaccines.

Methods Employed: Pigeons of different genetic stocks are inoculated with different strains of Rous sarcoma virus (RSV). Antibody to RSV is determined by the indirect immunofluorescence test and the direct complement-fixation test.

Major Findings: Previous investigators have demonstrated the usefulness of pigeon anti-Schmidt Ruppin serum in the direct complement-fixation test.

We found that different lots of the same RSV strain and different lots of other RSV strains can produce tumors varying from pea size to 40 mm in pigeons. The significance of tumor size to antibody production has not yet been ascertained.

Significance to Bio-Medical Research and the Program of DBS: The successful substitution of pigeon serum for hamster serum and chicken serum in either the immunofluorescence or the COFAL tests for detecting the group specific antigen of avian leukosis viruses of all subgroups would simplify testing for

avian leukosis viruses. This would be advantageous both to vaccine manufactureres and DBS.

Proposed Course of Project: To continue with the improvement and development of techniques for the detection of avian leukosis viruses in human viral vaccines of egg origin.

Honors and Awards: None

Publications: None

Serial No. 124
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on smallpox vaccines and vaccinia virus

Previous Serial Number: Same

Principal Investigator: Ruth C. Dunlap, Ph.D.

Cooperating Units: None

Man Years:
Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: (1) To determine the vaccinia virus particle content of smallpox vaccines by direct electron microscope counting procedures; (2) to investigate certain aspects of virus biology, using vaccinia virus as a model; (3) to study the interrelationship of various members of the poxvirus family.

Methods Employed: Standard techniques of viral propagation and assay have been used in conjunction with electron microscopy.

Major Findings: A system of enzyme treatment for smallpox vaccines has been devised which reduces the extraneous debris sufficiently that a direct electron microscope particle count of the virus can be made. This treatment does not reduce the infectivity of the virus nor cause actual destruction or visible degradation of the virus.

Further studies have been carried out concerning the effect of aggregation of vaccinia virus on biologic titer. Aggregation-dispersion experiments performed with smallpox vaccine virus, passed once in tissue culture to eliminate calf lymph debris, indicate that aggregation increases the infective titer.

Significance to Bio-Medical Research and the Program of DBS: The viral content of smallpox vaccines has always been assessed by indirect methods, involving infectivity for various hosts. The direct count of virus particles in the electron microscope provides a quick, easy quantitation which can then be used to correlate other methods of titration. Reduction of debris by enzyme treatment makes a direct count possible.

Proposed Course of the Project: The principal investigator and this

project were transferred to the Laboratory of Biophysics and Biochemistry (LBB), DBS in January, 1969. Interest in the improvement of quality of smallpox vaccines will be continued and studies of biological interrelationship of the poxviruses will be extended and future reporting will be from LBB.

Honors and Awards: None

Publications: None

Serial No. 59
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Antigenic studies of rickettsial vaccines

Previous Serial Number: Same

Principal Investigator: Richard A. Mason, D.V.M., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.4
Professional: 0.5
Other: 0.9

Project Description:

Objectives: Primary emphasis during this period was directed toward evaluating a candidate lot of inactivated typhus vaccine for use as a reference antigen. Studies of the immunogenicity and antigenicity of Rocky Mountain spotted fever rickettsia were postponed while the reference evaluation was in progress.

In a related study, the effects of different guinea pig immunization procedures were compared to the results seen with the standard guinea pig potency test.

Methods Employed: Candidate reference vaccine in final containers was evaluated on the basis of (1) the standard guinea pig potency test; (2) direct complement-fixation (CF) antigen titer and (3) antigenic potency in man. For the clinical trial a single 0.5 ml dose of vaccine was administered to 84 marine recruits, and antibody responses were determined on individual pre- and post-vaccination (28 days) serum samples by means of CF and toxin neutralization (TN) tests.

To study the effects of different guinea pig immunization regimens, groups of 15 animals were vaccinated once or twice (7 day interval) with undiluted, 1:10 or 1:100 dilutions of inactivated typhus vaccine. All guinea pigs were vaccinated subcutaneously with 0.5 ml of the appropriate vaccine dilution. Guinea pigs in each group were randomly divided into subgroups of 10 and 5. CF antibody responses were determined on individual serum samples and the mouse TN procedure was used to evaluated antibody levels in pooled

serum samples from five or ten guinea pigs.

Major Findings: The CF antigen titer of the candidate reference vaccine was 1:16 - 1:20. A titer of 1:40 for the reference was desired. The test vaccine had a guinea pig potency titer of 1:64 - 1:128. The seroconversion rate in man as determined by the CF test was 11% and 20%. The CF test procedure was inconsistent in detecting reactors at the 1:4 dilution level. The TN test used with human sera was more sensitive than the CF procedure for detecting early antibodies. Ninety-four percent (94%) of vaccine recipients had titers of 1:4 or greater 28 days after vaccination. This vaccine, although capable of evoking a good antibody response in man, was sub-optimal as a reference on the basis of its direct CF antigen titer and only marginally satisfactory with regard to its guinea pig potency titer. It was not selected as a reference reagent by the Commission on Rickettsial Diseases of the Armed Forces Epidemiological Board.

In the related potency test study it was found that regardless of whether guinea pigs were injected with one or two doses of vaccine (guinea pig potency titer for undiluted vaccine = 1:64), both the CF and TN antibody titers decreased as the dilution of vaccine increased. There was no evidence that the two dose regimen might, by potentiating the antibody response, result in acceptance of vaccines of widely different antigen contents. More extensive trials are required to settle this question. The relative decreases seen in TN antibody titers as the vaccine concentration was reduced were approximately the same regardless of whether one or two doses were used in vaccinating the guinea pigs. The CF and TN antibody titers following two injections of three different dilutions of vaccine were generally two-fold higher than titers resulting from single injections of the same dilutions. TN antibody titers were the same whether done on samples of serum pooled from five or ten guinea pigs, except in one instance where there was a two-fold difference in titer.

Significance to Bio-Medical Research and the Program of DBS: Selection of a reference typhus vaccine is essential as another means of insuring that only potent lots of new vaccine are approved and as a standard reagent for use in rickettsial research. Studies of the guinea pig potency test are directed toward determining possible variables that might affect the test results.

Proposed Course of the Project: This project will be continued during the coming year.

Honors and Awards:

Associate Member, Commission on Rickettsial Diseases, Armed Forces Epidemiology Board, Department of the Army, Washington, D. C.

Publications: None

Serial No. 65
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Mechanisms of intracellular infection

Previous Serial Number: Same

Principal Investigator: Richard A. Mason, D.V.M., Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 1.1
Professional: 0.3
Other: 0.8

Project Description:

Objectives: This is part of a continuing project designed to study biological and biochemical factors associated with intracellular parasitism. The present study was continued from last year as planned. The purpose of this phase of work is to define the nutritional requirements of the intracellular parasite Rickettsia quintana. Progress made will be useful in studies of more fastidious intracellular parasites.

Methods Employed: The ability of systematically simplified media to support growth of R. quintana was studied. Major efforts were directed toward defining essential growth factors in yeast extract and defining essential amino acids and/or vitamins.

Major Findings: When media were prepared with Ionagar instead of Difco Bacto-agar, yeast extract was not required for the optimal growth of R. quintana and previously masked requirements for amino acids and vitamins were revealed. With the Ionagar medium, the requirement for valine, one of the amino acids in Eagle's basal medium (BME), was extended to include all of the BME "essential" amino acids. With the BME "essential" amino acids present, the "non-essential" amino acids of BME were not absolutely required but did markedly enhance growth. Similarly, the nine vitamins of BME were not essential but enhanced growth over that seen with BME "essential" amino acids only. Glutamic acid was the only amino acid of the BME "non-essential" group found to be required for growth of R. quintana. Higher titers were seen, however, when the glutamic acid medium was supplemented with proline, another "non-essential" amino acid. Vitamins, previously beneficial but not

essential, became an absolute requirement for growth when only glutamic acid and proline were used instead of all of the BME "non-essential" amino acids.

Because conditions for propagating R. quintana are favorable for the growth of mold, much time has been lost in the past due to mold contamination. Consequently, trials were done to see if R. quintana could be grown in the presence of substantial levels of amphoteracin B. Amphoteracin B at concentrations of 3-12 mcg/ml had no influence on R. quintana titers. There was a 10% decrease from expected numbers of colonies associated with amphoteracin at 25 mcg/ml and 50% fewer colonies when the level of amphoteracin was increased to 50 mcg/ml.

Significance to Bio-Medical Research and the Program of DBS: Basic studies of host-parasite interaction are a necessary parallel to the development of systems useful for vaccine production and related testing procedures.

Proposed Course of the Project: This project will be continued during the coming year.

Honors and Awards: None

Publications: None

Serial No. 82
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of the nucleic acids and genetic relatedness of rickettsiae

Previous Serial Number: Same

Principal Investigator: Richard A. Mason, D.V.M., Ph.D.

Other Investigators: Emilio Weiss, Ph.D.
David T. Kingsbury, M.S.

Cooperating Units: Naval Medical Research Institute, NNMC, Bethesda, Md.

Man Years:
Total: 0.5
Professional: 0.2
Other: 0.3

Project Description:

Objectives: The project is designed to characterize rickettsiae with respect to their DNA base ratios and to compare the genetic relatedness of rickettsiae and other intracellular parasites.

Methods Employed: Rickettsial DNA's previously isolated from infected BS-C-1 cell culture were examined for differences in their base ratios as determined by thermal transition profiles. The size of the rickettsial genome was established from the DNA's of several species by ultracentrifugation analysis.

Major Findings: Data concerning the size of the rickettsial genome are presently being evaluated. Base ratios of several rickettsiae have been determined and others are still to be examined. In general, thermal transition values were in agreement with values determined earlier by chromatographic techniques but indicate distinct genetic differences between R. quintana and classical rickettsiae.

Significance to Bio-Medical Research and the Program of DBS: Studies of rickettsial genetics and DNA are of importance as an additional means of understanding relationships among rickettsiae and in discovering features in common between these microorganisms and other intracellular parasites.

Proposed Course of the Project: Homology trials planned for this fiscal year were not attempted but are scheduled for this summer when D. T. Kingsbury will return from California.

Honors and Awards: None

Publications: None

Serial No. 67
1. Virology & Rickettsiology
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Standardization of techniques applicable to large-scale production of cell cultures.

Previous Serial Number: Same

Principal Investigator: Henry C. Orr, Ph.D.

Other Investigators: Joseph L. Rogers
Nicola M. Tauraso, M.D.

Cooperating Units: None

Man Years:

Total: 1.0
Professional: 0.5
Other: 0.5

Project Description:

Objectives: (1) to develop new and improved techniques and methods for large-scale production of cell cultures, (2) to prepare cell cultures for use by DBS investigators, and (3) to maintain a collection of cell lines as a repository.

Methods Employed: Information regarding various techniques and methodology as related to large-scale production of cells is gathered from the literature and by consultation. This information is evaluated and applied in pilot studies to the production of large quantities of cells. Upon successful application, these improved and tested techniques are standardized as part of the Cell Biology Section procedures for producing quantity-cell cultures of high quality for research and vaccine testing purposes.

Major Findings: (1) A modified roller apparatus using the Wyble base unit was developed. The drum and bottles for this apparatus were made according to our specifications by the NIH machine and glass shops. Each bottle contains the equivalent of 6 32-oz. bottles. This apparatus is being used to grow and maintain the WI-38 cell stock, (2) a system of pretesting all tissue culture reagents before use was established in order to ascertain their growth promoting potential as an added quality control, (3) Fetal calf serum was found to be superior to calf serum inpromoting growth of RK-13, MA-104, and WI-38 cells, (4) the following cell lines were received, distributed on an experimental basis and stored in the Cell Culture Cell-Respository: $LLCMK_4$ (Squirrel

monkey kidney, Eli Lilly Co.); swine kidney; BRL-B3347 (diploid fetal rhesus monkey lung); opossum kidney (Microbiological Associates), (5) 20 lots of rabbit and bovine kidney cells were frozen in DMSO and stored in liquid nitrogen as control reference cells for the Division.

Significance to Bio-Medical Research and the Program of DBS: In order to assure reproducibility of results obtained by investigators in their control and research studies, standardization of our cell culture methodology and systems is of paramount importance.

Proposed Course of the Project: This effort will be continued during the coming year.

Honors and Awards: None

Publications: None

Serial No. 79
1. Virology & Rickettsiology
2. Cell Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Development of new cell culture systems as possible substrates for vaccine production and/or testing

Previous Serial Number: Same

Principal Investigator: Henry C. Orr, Ph.D.

Other Investigators: Edward B. Seligmann, Jr., Ph.D.

Cooperating Units: Laboratory of Pathology, DBS
Laboratory of Control Activities, DBS

Man Years:
Total: 0.8
Professional: 0.3
Other: 0.5

Project Description:

Objectives: To develop new established cell lines as additional substrates for vaccine production and/or testing.

Methods Employed: By using various cell culture techniques as may be appropriate, the isolation, passage, characterization and preservation of cell cultures from such seldom used tissues as brain, thymus, bone marrow, etc., of nonhuman origin are made.

Major Findings: Cells have been cultured from the thymus, spleen, liver, heart, and lung of rabbits and embryonic calves. In addition, cells from the spinal cord of African green monkeys, and cells from the liver and brain of rhesus monkeys have been initiated in culture. Cells from hamster brain tissue also were grown. The major effort this year, however, has been attempts to establish reptilian (snake) cells in culture and to study their similarities to and differences from mammalian cell culture systems. In this regard, cells from kidneys, embryos, heart, lung, muscle, liver and various glands were used. We have found that reptilian cells can be cultivated for 3-5 passages in certain sera at either room temperature 23-25 C or at 26 C. Embryonic and kidney tissues were the easiest to culture and consistently produced excellent growth. Of the mammalian sera tested, calf provided the best growth, and, in the case of sera from poikilothermic animals, snake serum was superior.

With exception of the snake cells, these experimental cells were distributed to various investigators for study.

Significance to Bio-Medical Research and the Program of DBS: Additional well-defined in vitro host systems are needed for vaccine production and for testing. In addition, there is a need for cell-host systems for a number of fastidious agents.

Proposed Course of the Project: To continue attempts to establish cells in culture from a number of organs from various animals. Collaboratively to examine these cells for adventitious agents; for their susceptibility to selected members of various virus groups, and to study the biology of the cells.

Honors and Awards: None

Publications: None

Serial No. 83
1. Virology & Rickettsiology
2. Cell Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on the biology of cultured cells

Previous Serial Number: Same

Principal Investigator: Henry C. Orr, Ph.D.

Other Investigators: James Baker
Peter G. Probst
Joseph L. Rogers

Cooperating Units: None

Man Years:
Total: 0.6
Professional: 0.3
Other: 0.3

Project Description:

Objectives: As a result of cross-fertilization between the research and production units in the Section, to carry out short-term studies on the biology of the cultured cell. This year we conducted studies on the retention of viability of trypsinized tissues kept at 4 C and the use of serum protein fractions in cell culture systems.

Methods Employed: Tools of the cell biologist such as cytochemistry, time-lapse cinematography, phase contrast and fluorescent microscopy are used in investigating these problems.

Major Findings: (1) Trypsinized cells from rhesus and vervet monkey kidneys and from chick and hamster embryo can be kept at 4 C for approximately one week and retain both viability and growth potential. The decline in viability was greatest in the case of the monkey kidney cells and least for hamster embryonic cells, (2) serum globulin fractions appear to be more effective than albumin in supporting cell growth. Cells from various species vary as to the amount of serum needed for growth with as little as 0.25% being adequate for monkey kidney cells. Of the many cell lines tested, WI-38 diploid cells appear to be the most sensitive system for testing cytotoxicity of serum fractions.

Significance to Bio-Medical Research and the Program of DBS: Information gained from studies in this project provides the direct and immediate basis on which changes in methodology are made in keeping with the goals of the

Section in producing high quality cell cultures. These results are directly applicable to cell culture production.

Proposed Course of Project: This is a continuing project and current studies are developed and investigated in the light of greater knowledge, need, and feasibility.

Honors and Awards: None

Publications: None

Serial No. 142
1. Virology & Rickettsiology
2. Cell Biology Section
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Identification of specific in vitro cell differential antigens

Previous Serial Number: Same

Principal Investigator: Henry C. Orr, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.2
Other: 0

Project Description:

Objectives: To demonstrate in cell cultures, specific antigen(s) which can serve as a marker for cell identification and characterization.

Methods Employed: Methods and techniques are employed to find a "clean" specific immunizing cell antigen. Such antigen to be used to obtain specific antiserum. These methods include isolation of various cell fractions by biochemical approaches, characterization of these fractions by physical and immuno-chemical techniques, preparation of antisera and subsequent localization and detection of cell antigens by serological techniques including immunofluorescence.

Major Findings: The WI-38 cell was used as the prototype. The following fractions have been obtained: (1) lysate residue, (2) lysate fraction, (3) cell "globulin" fraction, (4) cell "albuminoid" fraction. Antisera to these fractions were prepared in rabbits and tested for specificity and reactivity. In addition, the same cellular fractions were prepared from other human cell lines, i.e., KB, HeLa, FL, Chang's liver and HEp-2.

Although calf serum could not be detected by agar-gel diffusion tests in the respective fractions of WI-38 cells, the antisera to these fractions did contain antibodies to calf serum (a constituent of the culture medium). This contaminating antibody, however, could be removed by absorption. Antiserum to the "globulin" fraction reacted with all other cellular fractions. However, antiserum to the "albuminoid" fraction of WI-38 cells appeared to be fraction specific by gel diffusion tests. Although the antisera agglutinated WI-38 cells, it did not cause cell lysis.

Antisera to fractions of WI-38 cells have not, as yet, been tested with similar fractions obtained from the other human cells.

Significance to Bio-Medical Research and the Program of DBS: Cell culture typing by specific differential-markers is important from the point of view of detecting cross-cellular contamination and possible host cellular contaminants of vaccines produced in tissue cultures.

Proposed Course of the Project: Emphasis on this project will be increased. Furhter serological characterization will be made of the antisera and additional cell antigens, possibly cell surface antigens will be examined.

Honors and Awards: None

Publications: None

Serial No. 165
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies on immunologically active RNA (ribonucleic acid)

Previous Serial Number: None

Principal Investigator: Daniel E. Thor, M.D., Ph.D.

Other Investigators: S. F. Schlossman, M.D.

Cooperating Units: Harvard Medical School, Beth Israel Hospital, Boston, Massachusetts

Man Years:
Total: 1.1
Professional: 0.3
Other: 0.8

Project Description:

Objectives: Previous studies using human RNA species in vitro have shown the transfer of an immunological message to previously nonsensitive human lymphoid cells. This project is concerned with defining the mechanism of activity with the hope of evaluating RNA as a biologic product.

Methods Employed: Antigens were prepared by Dr. S. F. Schlossman as alpha (a), DNP-L-(lysine)$_{8-10}$ and epsilon (e), DNP-L-(lysine)$_{14}$ for use in tissue culture and immunization of inbred guinea pigs (strain 2 and 13). Guinea pigs were sensitized by foot pad injections, and initial immunity was determined by routine skin testing. Only those guinea pigs with positive skin test reactions were used for immune transfer responses using RNA extracts. The RNA was extracted from lymph nodes and spleens of sensitive and nonsensitive guinea pigs by a modification of the hot phenol method. The RNA extract was then incubated with nonsensitive lymph node cell suspensions and the cellular immune responsiveness determined in vitro by measuring cellular transformation with tritiated thymidine and production of migration-inhibition-factor (MIF), and in vivo by the ability to adoptively (passive) transfer the delayed hypersensitivity skin test reaction.

Major Findings: All strain 2 and no strain 13 guinea pigs form an immune response to DNP-oligo-lysines. Initial immunogen recognition is under genetic control and may depend on the existence of a specific receptor for the oligolysine molecule on an immunologically competent or "processing cell." Subsequent steps of immunogen recognition are ill defined but presumably

involve the establishment of committed cells with a specific recognition system for antigen and the capacity to synthesize mediators of the immune response. These studies show that RNA extracts from lymphoid tissue of strain 2 animals sensitized to chemically defined DNP-oligolysines can specifically transfer responsiveness to these antigens. Lymph node cells from nonimmunized strain 2 and nonresponder strain 13 guinea pigs were incubated with RNA and then exposed to DNP-oligolysines in vitro. Both strain 2 and 13 lymph node cells were rendered specifically sensitive. The response was measured by production of MIF in culture supernatants, by the cellular incorporation of tritiated thymidine, and by the ability to transfer the skin test to non-sensitive animals. RNA from nonimmunized guinea pigs or antigens alone were ineffective in transferring these responses.

Significance to Bio-Medical Research and the Program of DBS: Of major concern in studies of delayed hypersensitivity has been the development of an in vitro test, particularly for man, and the discovery of the factor or factors that confer a passive hypersensitivity state. This passive transfer with cells has often ended in a graft vs. host type reaction. RNA type materials may well demonstrate immunologic transfer without any deleterious effects in order to confer immunity to nonresponders (agammaglobulinemics, hypogammaglobulinemics) or offer solutions in the field of cellular immune responses (transplantation rejection).

Proposed Course of the Project: The ability to transfer diseases (such as experimental allergic encephalomyelitis) and disease protection will be studied. The recognition of radio-labeled antigen or antigen fragments in the RNA extract will also be explored.

Honors and Awards:

Invited program participant and discussant at the New York Academy of Sciences, Landsteiner Centennial, New York, New York. December 5, 6, 7, 1968.

Distinguished Service Award and Certificate of Merit Dictionary of International Biography, London, England, February 26, 1969.

Invited program participant and discussant at the International Symposium on Mediators of Cellular Immunity, Brook Lodge, Augusta, Michigan, April 28, 29, and 30, 1969.

Publications: None

Serial No. 166
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Immune reactions and immunochemistry of tissue culture and cellular antigens

Previous Serial Number: None

Principal Investigator: Daniel E. Thor, M.D., Ph.D.

Other Investigators: Richard A. Mason, D.V.M., Ph.D.
Henry C. Orr, Ph.D.
Nicola M. Tauraso, M.D.

Cooperating Units: None

Man Years:
Total: 0.8
Professional: 0.4
Other: 0.4

Project Description:

Objectives: This study is directed toward the isolation and immunological characterization of cell antigens by: (1) liberating these histocompatibility antigens from continuous culture cell lines, diploid cell cultures, or pooled inbred animal isologous cell types, (2) purifying and concentrating these antigens, and (3) identifying the cultures and cells from which the antigens originated by immunochemical techniques.

Methods Employed: Water soluble transplantation antigen(s) will be liberated and purified from available tissue cultures (BS-C-1, WI-38, MA-104) using laboratory techniques such as sonication, papain digestion, ultracentrifugation, column chromatography, disc electrophoresis. The isolated antigens will then be evaluated for histocompatibility differences, immunochemical differences and binding or affinity differences and similarities. Immune antibodies will be tested for precipitins, cytotoxicity and the ability to stimulate or block lymphocyte responses such as blast-transformation, radioactive incorporation and migration inhibitory factor (MIF) production.

Major Findings: Although only preliminary studies have been started at this time presumptive evidence indicates rather striking differences in immunochemical patterns. Other workers have already suggested that such antigenic differences can be recognized in some inbred guinea pigs.

Significance to Bio-Medical Research and the Program of DBS: Histocompatibility antigens and cell-associated antigens obtained in a water soluble state offer many advantages over whole cells in regards to antigenic identification. Some obvious examples are (1) identification of tissue culture variability and antigenicity, (2) control and standardization of transplantation immunology, (3) selectivity and potency of antilymphocyte globulin, (4) tumor cell identification and standardization, and (5) a better understanding of autoimmune disorders.

Proposed Course of the Project: Preliminary studies already suggest apparent differences in tissue culture antigens and the need for: (1) initial isolation of larger quantities of cell antigens, (2) purification of these antigens, (3) production of immune antibodies against them, and (4) further immunochemical characterization of the antigenic component.

Honors and Awards: None

Publications: None

Serial No. 167
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Immune responsiveness in man to viral agents

Previous Serial Number: None

Principal Investigator: Daniel E. Thor, M.D., Ph.D.

Other Investigators: Lewellys F. Barker, M.D.
Sheldon M. Wolff, M.D.

Cooperating Units: Laboratory of Clinical Investigations, NIAID
Laboratory of Viral Immunology, DBS

Man Years:
Total: 1.1
Professional: 0.3
Other: 0.8

Project Description:

Objectives: Cellular mediated immune phenomena (delayed-type-hypersensitivity) play an important role in the pathogenesis and in immune protection of many viral diseases. The purpose of this project is to evaluate the relative importance of cellular and humoral factors in determining individual responses to revaccination. If one or more in vitro procedures are found to be reliable indicators of immunity to challenge with vaccinia virus, the task of evaluating changes in the routine practice of smallpox vaccination would be aided considerably. The project was also designed to compare the cellular and humoral immune responses to revaccination reactions.

Methods Employed: Volunteers were vaccinated in the left deltoid area with a commercial lot of calf lymph grown vaccinia virus. The subjects were vaccinated similarly in the right deltoid area with an aliquot of the same vaccine lot which had been inactivated by heating for 30 minutes at 60 C. Skin reactions were read 1, 2, 3, 5, 7, 10 and 14 days after vaccination. All subjects who had equivocal reactions after the initial vaccination were revaccinated and the skin reactions observed as described above. Heparinized and clotted blood were obtained initially, 28 days after the first vaccination, and 28 days following the second vaccination in those who had been revaccinated.

Hemagglutination-inhibiting and plaque neutralizing antibody titers were determined on all serum samples. Cellular immune status was determined by current tissue culture methods of incubating peripheral blood lymphocytes

separated from the heparinized blood samples with vaccinia antigens. The lymphocytes will be studied for blast transformation, radioactive incorporation of tritiated thymidine and liberation of migration-inhibition-factor (MIF) into the supernatant fluids.

Major Findings: Following revaccination, volunteers with major skin reactions to vaccinia demonstrated: (1) significant increases in lymphocyte thymidine incorporation compared to pre-immunization levels of reactivity and (2) the appearance of antigen specific MIF production. Other serological and dose-antigen response data are not yet available.

Significance to Bio-Medical Research and the Program of DBS: For many years there has been considerable interest in reducing the morbidity and complications associated with smallpox vaccination. Approaches under investigation include further attenuation of the virus, primary vaccination with inactivated virus, vaccination accompanied by gamma globulin administration and administration of vaccinia virus by the subcutaneous route for primary immunization. The factors necessary for protective immunity against smallpox have not been well defined. Resistance to challenge by percutaneous revaccination has been the traditional method for determining the immune status of individuals. Levels of hemagglutination-inhibiting and plaque neutralizing antibodies do not appear to be reliable measures of immunity to revaccination. Although cellular immunity may play an important role, there is very little precise data available on this point. It is of prime importance, therefore, to be able to evaluate the cellular immune response as compared to antibody formation. This project should also add much needed correlates to other viral infections such as mumps, measles, and rubella.

Proposed Course of the Project: When all antibody and cellular immune data are available it should be possible to evaluate virus antigen responsiveness and vaccination reactivity. If these are helpful, additional studies will be conducted with other viruses.

Honors and Awards: None

Publications: None

Serial No. 60
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies concerned with influenza viruses

Previous Serial Number: Same

Principal Investigator: J. A. Morris, Ph.D.

Other Investigators: None

Man Years:
Total: 1½
Professional: 3/4
Other: 3/4

Project Description:

Objectives: Work on this project was directed toward collection of information from many biologic study systems which will be of assistance in improving effectiveness of respiratory virus vaccines.

Immunologic preparative effect of small doses of vaccine and studies on influenza in immunologic virgin populations.

Influenza virus A. Isolated Pacific island populations without earlier exposure, or a limited and defined history of exposure to influenza confirmed by serologic testing, were immunized in 1965 and 1966 with one of four inactivated monovalent influenza A2 vaccines.

In people never before exposed to influenza, a single dose of vaccine resulted in the production of low levels of homologous antibody in approximately three-fourths of the vaccinees, with a variable but always considerable degree of cross-reaction among other strains of the A2 virus family, but virtually no cross-reaction with the A1 and A virus families. Many individuals demonstrated a delayed antibody response, with higher titers at 7 months than 3-7 weeks after vaccination, again depending on the vaccine used, but amounting in the aggregate to one-fifth of homologous and nearly one-half of the heterologous A2 antibody responses. People with an earlier exposure to

either epidemic influenza type A1 or A showed positive homologous responses after a first dose of vaccine in 90 percent of the vaccinees, a greater degree of cross-reactivity among strains of the A2 virus family than was seen in the virgin group, and a pronounced recall of antibody to the previously infecting virus subtype as well as cross-reaction to the other early influenza virus subtype. Delayed rises of both homologous and heterologous A2 antibody in such previously exposed people were much less frequent than in the virgin group. The combination of 2 doses of A2 vaccine given several months apart yielded antibody levels in people never before exposed to influenza virus as high and broad as in people who had been exposed to earlier virus subtypes, irrespective of the presence of detectable pre-booster antibody; in people previously exposed to influenza A2 infections, a comparably intense response was observed after a single dose of A2 vaccine. Finally, primary response to natural infection induced a much greater antibody response than did primary vaccination among immunologically virgin people of the same general population, but the pattern of cross-reactivity was qualitatively similar.

Influenza virus B. Populations of several isolated Pacific islands for whom historical information and serological testing had yielded a precise knowledge of earlier exposure, or lack of exposure, to influenza were immunized with one of two inactivated monovalent influenza virus type B vaccines, containing either the Great Lakes 1739/54 or Maryland/1/59 strains. Eighty percent of people without prior exposure to influenza failed to respond with neutralizing antibody after a single dose of vaccine; the remainder responded at very low levels. A booster injection, given 7 months after the initial dose, uniformly produced a 4-fold or greater rise in homologous antibody, with comparable but usually somewhat lesser rises to all tested heterologous strains, except the distantly related Lee/-/40 strain, to which little or no response occurred. A quite different response pattern was seen in people with a single natural infection 15 years before with influenza type B: in them the greatest response occurred following the first dose of vaccine, with little or no rise after the booster.

Five months after the booster vaccination an epidemic of influenza type B occurred in one of the island populations studied. The attack rate was approximately 80 percent. Incidence of infection, in terms of either the occurrence of the commonest symptom, cough, or a significant rise in antibody titer, was not reduced in the segment of the population given type B vaccine when compared with a control group given type A vaccine. Modest reduction in the severity of acute disease in the Great Lakes vaccine group was apparent in a lower incidence of fever

and pharyngitis, and use of both type B vaccines was associated with a reduction in the occurrence of otitis media, particularly in school children.

A full description of influenza virus work in Micronesian populations is found in a report by Dr. D. C. Gajdusek (NINDS) and in 2 manuscripts prepared for publication.

Attempts to determine the antigenic structure of the 1918 epidemic influenza virus. Because the strain of virus responsible for the 1918 influenza pandemic was not recovered at the time, a precise statement about its antigenic composition probably will never be made, but considerable evidence has since suggested it to be most closely related to the virus of swine influenza first isolated in 1931.

In 1964, we discovered that the population of one particularly inaccessible Pacific island had not experienced infection with type A influenza in over 40 years, and thus had an opportunity to circumvent the entire issue of cross-reactivity from multiple infection. Specifically, in a total population of 200, not a single individual under the age of 40 who had never been off the island had detectable neutralizing antibody to any of several strains of virus spanning the recognized subgroups of type A influenza, whereas antibody to the early type A human isolates was present in approximately half of the 80 people born before the epidemic who were still alive and bled in 1964. We chose for our initial analysis 10 individuals who were born in the years just before the epidemic, and so would have been most unlikely to have experienced any other than this single infection. None of the sera contained neutralizing antibody to representative influenza viruses A1 and A2, but all contained antibody to A/PR/8/34, A/WS/-/33 and A/Swine/1976/31 with higher titerd directed against A/PR/8/34 than against the other 2 virus strains. Accordingly, it appears that the virus responsible for the 1918 pandemic was more closely related to A/PR/8/34 than to the virus of Swine influenza, as was previously believed.

Viruses resistant to the action of chemical and physical agents. The aspects of this study concerned with the pathology induced by the scrapie agent in experimentally infected mice was successfully concluded and the findings were published. The aspects of the study concerned with the enhancement of the scrapie process in mice by the use of dimethylsulfoxide (DMSO) and Mycoplasma are in progress.

Studies on viruses recovered from and capable of producing in man illnesses indistinguishable clinically from influenza. Pellissier and Lumaret in 1948 reported the recovery in guinea pigs of a transmissible agent from the blood of a patient with African epidemic icterus and subsequently these workers recovered strains of the same agent from patients with an anicteric, influenza-like, type of the disease. It was previously reported that on the basis of serologic data that it appeared that African epidemic icterus virus (AEIV) was a strain of lymphocytic choriomeningitis (LCM) virus. In order to determine the etiologic importance of this virus in the illness African epidemic icterus, we obtained from the Pasteur Institute in Bangui paired and serial sera from recent cases of African epidemic icterus. In none of the patients were we able to demonstrate antibody rises to the AEIV strain of LCM virus. Accordingly, it appears possible that the virus sent to us for study by Pellissier represents a laboratory contaminant. A statement concerned with these findings is being prepared for publication.

Characterization of viruses and other microbial agents encountered in test animals and in cell cultures employed in testing of biologics. Prior to 1968 four viruses (virus III, papilloma, myxoma and rabbitpox) were known to occur in laboratory rabbits. In 1968, in collaborative work with the Atomic Energy Commission, a study was begun to determine the cause of an epizootic in cottontails in Kansas in which we used as experimental host in virus isolation work, cultures of primary laboratory-rabbit-kidney-cells. In the course of this work we recovered in uninoculated rabbit-kidney-cells three viruses previously not known to occur in domestic rabbit: rabbit syncytium, rabbit vacuolating and another unknown, and previously undescribed virus, rabbit foamy virus. In addition, in collaborative work with the National Cancer Institute, we found in the experimental host cells, an agent that induces typical herpes-like intranuclear inclusions. The latter agent in all possibility is virus III, which, until recently had not been reported in rabbits since 1940. Thus, the domestic rabbit is not, as was recently thought, relatively free of viruses. Little or nothing is known of the behavior in man of viruses indigenous to the rabbit. Until such information is available, caution is essential in the use of rabbit kidney cells in the production of vaccines destined for use in man.

One of the three viruses (rabbit syncytium virus) was intensively studied by electron microscopy. In this work, it was noted in electron scanning work by our collaborators at

the Oak Ridge National Laboratory, that except for similarities to changes induced by the virus of epidemic diarrhea of infant mice, rabbit syncytium virus induces in infected cells cytoplasmic and nuclear changes that are unlike those of any known virus. These changes are described in detail in an interpretative report prepared for publication.

It was reported previously in a published account (Proc. Soc. Exp. Biol. and Med. 129: 489-494, 1968) that a new species of Mycoplasma was recovered from scrapie infected sheep and mouse brains and from cell cultures derived from human, chimpanzee and dog tissues. The study which resulted in this recovery was undertaken to determine whether Mycoplasma were present in the brains of scrapie infected animals and in the brains of man with kuru and was prompted by similarities in the clinical manifestations of scrapie in sheep, goats and mice, kuru in man and M. neurolyticum disease in mice. Absence of Mycoplasma in 8 kuru brains and their infrequent recurrence in brains of scrapie sheep (2 of 19) are indications that Mycoplasma are probably not associated etiologically with kuru or scrapie. Nevertheless, the role the new Mycoplasma plays in the ecology of its ovine and murine hosts is unknown and is the subject of current studies. The present interest in the new Mycoplasma rests in the observation that it is a common, troublesome, cell culture contaminant; its presence interferes with the use of the cells in virus and other studies.

Proposed Course of the Project: Despite 30 years of effort, little is known of the immune processes by which man is protected against infection with influenza viruses. Even so basic a conception as Francis'"doctrine of original antigenic sin" is now modified as a result of the information presented in this report. Additionally, we present information in this report derived from our Micronesian experience that shows that disappointingly little protection against infection resulted in the study population following use of influenza virus B vaccine. Thus, efforts will continue to be made to accumulate information which might be of use in improving the effectiveness of influenza virus vaccines.

Laboratory differentiation of influenza and influenza-like-illnesses is an essential continuing study. Continuation of this study will make it possible to accumulate information that will be of assistance in eliminating or, at least, in reducing in importance a complicating factor in interpretation of influenza virus vaccine protection data and of other epidemiological information related to influenza in man.

The definitive characterization of transmissible agents encountered in the tissues of animals used in the production or testing of vaccines will continue to be of assistance in overcoming manufacturing problems and in defining variables which are inherent in vaccine potency assay systems.

Honors and Awards: None

Publications:

1. Barile, M. F., DelGiudice, R. A., Carski, T. R., Gibbs, C.J., and Morris, J. A.: Isolation and characterization of Mycoplasma arginini. Proc. Soc. Exp. Biol. & Med. 129:489-494, 1968.

and

2. Brown, P., Gajdusek, D. C.,/Morris, J. A.: Antigenic response to influenza virus in man. I. Neutralizing antibody response to inactivated monovalent A2 vaccine as related to prior influenza exposure. Am. J. Epidem. In Press.

3. Brown, P., Gajdusek, D. C., Chen, K. M., and Morris, J. A.: Antigenic response to influenza virus in man. II. Neutralizing antibody response to inactivated monovalent B vaccine, with observations in vaccine efficacy during a subsequent type B epidemic. Am. J. Epidem. In Press.

and

4. Brown, P., Gajdusek, D. C.,/Morris, J. A.: The virus of the 1918 influenza pandemic: New eivdence about its antigenic character. Science. In Press.

Serial No. 98
1. Virology & Rickettsiology
2. Virology & Rickettsiology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies concerned with adenoviruses

Previous Serial Number: Same

Principal Investigator: J. A. Morris, Ph.D.

Other Investigators: Walter Dowdle
Bernice Eddy, Ph.D.
Robert Garman
Frances Judge
Wendell Niemann
Mehmet Saglam

Cooperating Units: National Communicable Disease Center
Laboratory Aids Branch, DRS
Medical Command, Turkish Army (Ankara)

Man Years:
Total: 1/2
Professional: 1/4
Other: 1/4

Project Description:

Objectives: The objective of this project is the accumulation of information which will be of help in re-establishing adenovirus vaccines to the armamentarium of respirovirus vaccines for use in man, and in addition, will be of assistance in understanding the biology of adenovirus infection.

Serologic and clinical responses in man and animals to viral components in adenovirus vaccines. Pedreira et al (Proc. Soc. Exp. Biol. and Med. 129:472-477, 1968) searched unsuccessfully "for an animal model for human adenovirus studies". The search was limited to the rhesus monkey, an animal already reported to undergo only an inapparent infection following exposure to human adenoviruses. In our work, we found, largely by accident, such a model, the chimpanzee. Moreover, the use of the chimpanzee for human adenovirus work, does not impair the utilization of this animal in the study for which it was originally purchased--in the present instance, in the study of kuru. In April-May 1968, an outbreak of coryza occurred in recently acquired chimpanzees in the animal quarantine facility at the National Institutes of Health. From

one of the sick chimpanzees we recovered human adenovirus type 31. The donor chimpanzee as well as other chimpanzees involved in this, the first of two outbreaks, developed specific antibody against the recovered virus in the weeks following the epizootic. Another group of chimpanzees introduced into the quarantine facility about 4 weeks after the termination of the first episode developed clinical coryza and subsequently experienced antibody rises to the recovered as well as to the prototype strain of human adenovirus type 31. These findings are of importance not only because they provide an animal model for the study of human adenovirus infections, but because they necessitate modification in the widely held belief that "man provides the only reservoir of adenovirus that infect humans." This belief is no longer tenable.

The aspects of this project which was concerned with an improved adenovirus type 4 vaccine was successfully concluded and a report is in preparation for publication. The aspect of the project concerned with the serologic and clinical responses in hamsters and monkeys to simian adenovirus 20 and to SV_{20}-SV_{40} complexes are still in progress.

Recovery of adenoviruses from Turkish Army recruits with acute respiratory illness. Adenovirus type 6 infection in man usually results in conjunctivitis, only rarely is it associated with respiratory disease. A recent respiratory disease outbreak in recruits in the Turkish army was etiologically associated with adenovirus type 6. A report of this infrequent etiologic association is in preparation for publication in a Turkish medical journal.

Proposed Course of the Project: As with influenza, little information is available on the basic biology of the behavior of adenoviruses in man and animals. The work of the past year illustrates this point. Previously, it was not known that an animal other than man could serve as a reservoir of adenoviruses capable of causing disease in humans. We now know that there is such an animal, the chimpanzee. Studies to collect and to interpret information concerned with the biology of adenoviruses will continue.

Honors and Awards: None

Publications: None

Contract Narratives

CONTRACT NARRATIVES

Table of Contents

CONTRACT NARRATIVES

		Page
Allergenic Extracts	Development of Information on the Activity of Allergenic Extracts (NIH-69-2010)	289
Beta-Propiolactone	Investigation of the Reaction Products of Beta-Propiolactone (PH-43-67-1397)	277
Carcinogenesis	Study of Carcinogenic Potential of 7 Chemicals Used in Biological Preparations (PH-43-67-677) (PH-43-67-676) (PH-43-67-684)	264
Chem. Carcinogenesis	Investigations on the Mechanisms of Chemical Carcinogenesis (PH-43-67-675)	262
Diploid Cell Strains	Development of Sub-Human Primate Diploid Cell Strains and Established Cell Lines (NIH-69-98)	287
Diploid Cell Strains	Development of Sub-Human Primate Diploid Cell Strains (NIH-69-100)	288
Electron Microscope	Electron Microscope Services (PH-43-67-1105)	274
Electron Microscopy	Electron Microscopy Services (NIH-69-2013)	290
Exchange Transfusions	Studies of Patients Receiving Exchange Transfusions in Neonatal Period (PH-43-67-701) (PH-43-67-705)	268
House Dust	Identification, Isolation, and Culture of Mites from House Dust (NIH-69-65)	285
Human Karyotypy	A Statistical Approach to Human Karyotypy (PH-43-67-1463)	278
Inf. Mononucleosis	True Incidence of Infectious Mononucleosis in Science (NIH-69-85)	286
Latent Infection	Studies of Latent Infection and Tissue Culture Phase (PH-43-67-1106)	275
Latent Infections	Studies of Latent Infections: Tissue Culture and Animal Phases (PH-43-67-1100)	270
Measles Vaccine	Study of Recipients of Attenuated Measles Virus Vaccine Prepared in Canine Kidney Cells (PH-43-68-646)	282

		Page
Mycoplasma Antigens	Preparation of Mycoplasma Antigens and Antisera (PH-43-66-1163)	261
Oil Adjuvants	Studies on the Biologic Activity of Oil Adjuvants (PH-43-67-679)	266
Oils & Emulsifiers	Chemical and Physical Analysis of Oils & Emulsifiers (PH-43-67-1101)	272
Oncogenic Activity	Studies of the Oncogenic Activity of Human Biological Products in Syrian Golden Hamsters (Mesocricetus auratus) (PH-43-67-1483)	280
Plastic Containers	Effects of Storage Conditions on Plastic Blood Containers (PH-43-65-1046) (PH-43-68-97)	259
Poison Ivy	Isolation of the Active Principles of Poison Ivy, Oak, Sumac, and other Plants of this Family and the Synthesis of Substances Related to the Active Principles (PH-43-64-76)	256
Poison Ivy	Determination of Potency and Efficacy of Urushiol, the Active Ingredient of Poison Ivy Extract (PH-43-64-885)	258
Poison Oak	Determination of Potency and Efficacy of the Active Ingredient of Poison Oak Extract by Clinical Studies (PH-43-68-632)	281
Pollen Extracts	Studies of the Biochemical Properties of Pollens and Pollen Extracts (PH-43-68-1250)	284
Rhesus Monkeys	Housing and Maintenance of Rhesus Monkeys (PH-43-68-662)	283

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

Allergenic Products Section

COLUMBIA UNIVERSITY (PH-43-64-76)

Title: Isolation of the Active Principles of Poison Ivy, Oak, Sumac, and other plants of this family and the Synthesis of Substances related to the Active Principles

Contractor's Project Director: Prof. Charles R. Dawson

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Henry Godfrey, M. D.

Objectives: (1) To obtain the active principles of poison ivy, poison oak, and poison sumac. (2) To determine whether these substances are present in commercial preparations. (3) To synthesize the active principles and related substances.

Methods Employed: Various isolation, synthetic, and analytical methods are employed.

Major Findings: 1. The pure urushiols from poison ivy and oak were prepared in sufficient quantity for clinical studies. 2. The urushiols of poison oak and sumac are different from that of poison ivy. Poison oak urushiol has a 17 carbon side-chain while poison ivy urushiol has a 15 carbon side-chain. The complete structure of the poison oak and sumac urushiols has not yet been determined. 3. The four components of poison ivy urushiol have been supplied in pure form and are being studied. 4. The thin layer chromatographic assay for the catechols has been developed and is being studied by DBS. 5. A start has been made on studying protein conjugates of catechols, and also the plant enzymes that inactivate catechols.

Significance to DBS and Bio-Medical Research: This project will provide information on a group of licensed products, Poison Ivy Extract, Poison Oak Extract, and, eventually, Poison Sumac Extract, and related products that are potentially subject to license, i.e., Cashew Nut Oil Extract. The chemical composition of the active ingredients will be determined and the degree of immunologic relationship will be examined to determine whether they might be used interchangeably. The synthetic substances will be used to examine the relationship of chemical structure to ability of catechols to sensitize and to determine whether some of them may be

substituted for the naturally occurring catechols for diagnosis or treatment of poison ivy, oak, or sumac sensitivity.

Proposed Course of Project: To continue until objectives are reached. The current contract will expire December 1969.

Date Contract Initiated: November 18, 1963

Current Annual Level: $20,000

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

Allergenic Products Section

ORENTREICH RESEARCH CORPORATION (PH-43-64-885)

Title: Determination of Potency and Efficacy of Urushiol, the Active Ingredient of Poison Ivy Extract

Contractor's Project Director: Robert Auerbach, M. D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Henry Godfrey, M. D.

Objectives: (1) To determine whether poison ivy extract or the active principles can hyposensitize poison ivy sensitive humans. (2) To ascertain whether the administration of these extracts to humans causes any toxic symptoms.

Methods Employed: The pure urushiol was prepared from poison ivy leaves by Prof. Charles R. Dawson at Columbia University under Contract (PH-43-64-76) and was prepared for clinical use by the Pharmaceutical Development Service of NIH. A commercially-obtained poison ivy extract was standardized at DBS by guinea pig potency assay and then supplied to the contractor. The contractor administered these materials parenterally and orally. Skin tests prior to and during treatment were used to assess the efficacy. Blood and urine samples were taken at regular intervals for laboratory examination to aid in determining whether any toxic effects were found.

Major Findings: Due to the inability of the contractor to obtain a sufficient number of sensitive individuals, no definitive findings were obtained. The limited data suggest that some desensitization occurred. Among those patients that were treated, laboratory studies indicated that no changes occurred in blood chemistry or morphology or urine chemistry suggesting that for the period of time administered the product is safe.

Significance to DBS and Bio-Medical Research: Although the contractor was unable to establish the efficacy of treatment of poison ivy sensitivity, he was at least able to demonstrate that over the time interval of treatment employed that there were no harmful effects attributable to the treatment.

Proposed Course of Project: Due to the inability of the contractor to obtain a sufficient number of patients, the contract will not be renewed.

Date Contract Initiated: May 26, 1964

Current Annual Level: $8300

CONTRACT NARRATIVE
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

BAYLOR UNIVERSITY (PH43-65-1046)

UNIVERSITY OF TEXAS (PH-43-68-97)

Title: Effects of Storage Conditions on Plastic Blood Containers

Contractors' Project Directors: Dighton Rowan, Ph.D.
Wallace L. Guess, Ph.D.

Project Officers (DBS): Madge L. Crouch, LBBP
Clarence F. Szwed, LBBP

Objectives: To determine the effects of storage time and temperature on plastic blood containers of different manufacturers, in order to evaluate the necessity for specifying dating periods and storage conditions for these containers.

Methods Employed: Extracts of the ACD anticoagulant and samples of stored whole blood and plasma were subjected to physical-chemical and biological tests. The toxicity of the extracts was studied through intravenous injection of mice and the intradermal injection of rabbits. In addition, *in vivo* cell survival studies were conducted. These studies were conducted as a cooperative project between these two contractors.

Major Findings: Extractants of stored contents of polyvinyl chloride blood-collecting containers revealed that samples from certain containers consistently exhibited toxic biological effects on test animals when injected intravenously or intradermally, and all containers caused demonstrable evidence of toxicity to cells in culture. Physical-chemical examinations of the extracts suggest that the limit of solubility of these extracts is reached sometime between sterilization and the three-month storage period, and although the total amount remains essentially constant, thin-layer chromatography denotes a change in the composition of the extractive materials. The presence of organotin in one group of containers and the consistent evidence of jaundice in mice injected with extracts from these containers suggests that one or a combination of extractive materials could serve as a predisposing agent to liver damage by organotin.

Significance to DBS Program and Bio-Medical Research: Because of the wide-scale use of plastic containers for blood and blood products, the definitive data compiled should be useful in determining if specifications on formulations, dating, and storage conditions are indicated in order to assure the safety, purity and potency of the contents.

Proposed Course of Project: These contracts were completed August 27, 1968 and October 22, 1968, respectively.

Date Contract Initiated: Baylor University-June 28, 1965
University of Texas-March 30, 1964

Current Annual Level: The total amount funded for these two contracts was $413,510.

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1969

Section on Viral Immunology

HUNTINGDON RESEARCH CENTER (BBL) (PH-43-66-1163)

Title: Preparation of Mycoplasma Antigens and Antisera

Contractor's Project Director: Theodore R. Carski, M.D.

Project Officers (DBS): Hope E. Hopps, LVI
Michael F. Barile, LBP

Objectives: (1) To prepare standardized Mycoplasma antigens and antisera which will be available for use by the Laboratory of Bacterial Products and other research groups within the DBS; (2) to provide a testing service for detection of Mycoplasma in biological materials being used in the Division's various programs, e.g., tissue cultures, virus and rickettsial seed pools, sera, etc.; and (3) provides services for isolation and identification of Mycoplasma from specimens obtained from other DBS Contracts (PH-43-67-1100 and PH-43-671106).

Methods Employed: (1) For preparation of antigens the strains will be thrice-cloned, certified and grown in suitable media. (2) Antisera are prepared in burros and donkey. Pre- and post-immunization sera (lyophilized) as well as fluorescent antibody will be available.

Major Findings: (1) The DBS has received serum specimens from animals immunized with all of the "human" strains and 25 "animal" strains. Conjugated serum for immunofluorescence studies has also been made available. (2) During the period from September 28, 1968 to March 28, 1969, 585 specimens have been tested for presence of Mycoplasmas.

Significance to DBS Program and Bio-Medical Research: The Mycoplasma constitute an important group of organisms, both with immediate regard to the regulatory control of vaccines and to their overall significance in human disease.

Proposed Course of Project: The termination date is September 28, 1969. Recommendation for renewal will be considered.

Date Contract Initiated: September 28, 1966
Amended September 28, 1967
Renewed September 28, 1968

Current Annual Level: $104,920.

CONTRACT NARRATIVE
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

UNIVERSITY OF OREGON MEDICAL SCHOOL (PH-43-67-675)

Title: Investigations on the mechanisms of chemical carcinogenesis

Contractors' Project Director: Dr. A. Wesley Horton

Project Officer (DBS): Dr. Pinya Cohen, LBBP

Assistant Project Officer (DBS): Dr. M. Carolyn Hardegree, LBP

Objectives: Develop short-term biophysical tests that correlate with existing long-term biological test systems designed to detect chemical carcinogenic activity; apply such tests to adjuvants components and their fractions; conduct studies on the composition of mineral oil; and determine the effects of carcinogens on the immunologic response of guinea pigs and mice.

Methods Employed: Comparative biophysical measurements are taken of both standard, known chemical carcinogens and various adjuvant components and their fractions. Components and fractions which yield data similar to known carcinogens are subjected to confirmatory animal tests.

Studies on the composition of mineral oil employ high efficiency vacuum distillation and liquid thermal diffusion.

In vivo immunoblast responses in guinea pigs and in vitro transformation of guinea pig lymphocytes are being used to evaluate adjuvant activity.

Major Findings: Three volatile fractions of mineral oil actively promote carcinogenesis by known chemical carcinogens in mice. This activity correlates with their ability to retard ion transport in a biophysical model which simulates a cell membrane. This model system shows promise as a quick screening test for chemical carcinogens.

Arlacel A enchances the skin test response in guinea pigs to contact sensitizers.

Liquid thermal diffusion has effectively separated components of narrow distillate fractions from mineral oil.

Significance to DBS Program and Bio-Medical Research: These studies may accelerate the development of quick, in vitro tests to determine the presence of carcinogens in components used to prepare adjuvants; elucidate mechanisms of action of carcinogens at the cellular and subcellular level; and establish criteria for the safety of adjuvant preparations.

Proposed Course of Project: The contract will continue through March 31, 1970.

Date Contract Initiated: March 17, 1967

Current Annual Level: $72,500

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

BIO-RESEARCH CONSULTANTS (PH-43-67-677)
MASON RESEARCH INSTITUTE (PH-43-67-676)
WARF INSTITUTE, INC. (PH-43-67-684)

Title: Study of Carcinogenic Potential of 7 Chemicals Used in Biological Preparations

Contractor's Project Director:

Bio-Research Consultants - Dr. Freddy Homburger
Mason Research Institute - Dr. Marcus Mason
WARF Institute Inc. - Dr. Paul Nees

Project Officer (DBS): Dr. Stanley H. Singer

Objectives: To test the oncogenic potential and co-carcinogenic activity of the following 7 compounds found in biological products as preservatives, inactivating agents, stabilizers, or additives: thimerosal, benzethonium chloride, parabens, phenol red, pyridine, ethylene glycol, and ethylene chlorohydrin.

Methods Employed: The compounds are injected via various routes into several species and ages of animals. Methods designed to accelerate and enhance detection of any carcinogenic potential include: pooling of injection sites with reintroduction into secondary homologous hosts, the use of a sensitive indicator animal, and co-carcinogen studies.

Major Findings: The results of the testing in mice have been negative to date indicating no evidence of carcinogenic or co-carcinogenic activity. Preliminary, but as yet incomplete, data from rat studies suggest that one of these compounds may be involved with a higher than expected level of tumor incidence.

Significance to DBS Program and Bio-Medical Research: These programs constitute part of the continuing effort to assure that various components of biological products are free from carcinogenic or co-carcinogenic activity.

Proposed Course of Projects: The contract with Bio-Research Consultants terminated August 22, 1968. The contract with Mason Research Institute is scheduled to terminate on April 30, 1969, and the contract with WARF will terminate on April 26, 1969.

Dates Contracts Initiated:

Bio-Research Consultants - May 23, 1967

Mason Research Institute - February 16, 1967
WARF Institute, Inc. - April 27, 1967

Current Annual Levels:

Bio-Research Consultants - Terminated
Mason Research Institute - $71,150
WARF Institute, Inc. - $28,892

CONTRACT NARRATIVE
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

MIDWEST RESEARCH INSTITUTE (PH43-67-679)

Title: Studies on the biologic activity of oil adjuvants

Contractors' Project Director: Dr. John O. MacFarlane

Project Officer (DBS): Dr. Pinya Cohen, LBBP

Assistant Project Officers: Dr. M. Carolyn Hardegree, LBP
Dr. Carl E. Miller, OD

Objectives: Determine the safety of oil adjuvants with regard to oncogenic or other pathologic activity.

Methods Employed: Groups of inbred and random bred mice are injected subcutaneously with mineral oil, peanut oil, Arlacel A emulsifier, aluminum monostearate and antigen, singly, in mixture, and in emulsion. Other groups receive that same treatment plus a known chemical carcinogen. Gross and microscopic pathologic examinations of the mice are made over a 1 1/2 to 2 year period to determine the development and/or acceleration of tumors or other pathologic activity.

Major Findings: In the direct oncogenic tests with mineral oil, both singly and in mixture, a large number of fibrosarcomas have occurred in random bred Swiss mice observed for 90 weeks. Tumors have occurred earlier and more frequently in males than females. These oncogenic effects have not been seen in the two inbred mouse strains. Cocarcinogenesis studies showed that Arlacel A emulsifier promotes tumor induction in all strains and both sexes of mice.

In tests with peanut oil adjuvant no direct oncogenic effects have been observed through the initial 36 weeks, however, in cocarcinogenic studies these adjuvants plus carcinogen promote tumor induction 2 to 4 times that observed with carcinogen alone.

Significance to DBS Program and Bio-Medical Research: Evaluation of adjuvant preparations in animals under a variety of conditions will provide data on the safety of these materials. The results of this and other studies will provide a basis for decisions on the use and potential safety of oil adjuvants in man.

Proposed Course of Project: The contract will continue through December 31, 1970.

Date Contract Initiated: February 24, 1967

Current Annual Level: $370,730

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

UNIVERSITY OF PITTSBURGH (PH-43-67-701)
UNIVERSITY OF MINNESOTA (PH-43-67-705)

Title: Studies of Patients Receiving Exchange Transfusions in Neonatal Period.

Contractor's Project Director: Dr. Edward R. Schlesinger, Dr. Howard Turner, University of Pittsburgh
Dr. Allyn G. Bridge, University of Minnesota.

Project Officer (DBS): Dr. Stanley H. Singer

Objectives: These contracts are investigating whether the administration of exchange transfusions which may contain extraneous agents, such as latent viruses, results in any diseases in later life over and above control populations which did not receive exchange transfusions. Particular attention is being given to leukemia and other diseases of possible viral etiology. In addition, at the University of Pittsburgh, a search for chimerism in exchange transfused children is being made.

Methods Employed: A group of infants who received exchange transfusion and an appropriate control group have been identified via a search of hospital records. Health histories of these groups are being obtained via questionnaire or personal interview of parents and, in some cases, physicians. At the University of Pittsburgh analysis of chromosomes of newborns who received exchange transfusions are being performed as part of the chimerism study.

Major Findings: Preliminary data indicate no unexpectedly high incidence of neoplasia or other disease entity in exchange transfused children. Chimeras have been identified among the exchange transfused population at the University of Pittsburgh and have been found exclusively in those cases receiving intrauterine transfusions rather than those receiving transfusions after birth.

Significance to DBS Program and Bio-Medical Research: This program will help the Division to fully discharge its responsibilities for the safety of blood and blood products. In addition, although exchange transfusion is a common occurrence in current medical practice, there is a dearth of studies available in the literature which indicate the health status of these children several years after transfusion. This study will help fill this gap. The results of the chimerism studies are of significance in that they not only elucidate a possible side effect of intrauterine transfusions, but also may provide a significant lead toward the understanding of the development of human immune mechanisms.

Proposed Course of Project: Contract with the University of Minnesota is scheduled to terminate in June, 1969. It is anticipated that the contract with the University of Pittsburgh will be extended an additional year.

Contract Level: University of Pittsburgh - $59,844 for 12-month period.
University of Minnesota - $71,000 for 12-month period.

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

MELPAR, INCORPORATED (#PH-43-67-1100)

Title: Studies of Latent Infections: Tisue Culture and Animal Phases

Contractor's Project Director: Dr. John Verna

Project Officers (DBS): Dr. B. G. Young (Latent Viruses) and Dr. M. F. Barile (Mycoplasma)

Objectives: To investigate primary African green monkey kidney, canine kidney, duck embryo and a continuous line of human lung fibroblasts (WI-38 cells) for covert oncogenic and/or other adventitious covert viruses and for Mycoplasma infection.

Methods Employed: Primary cultures of the above cells and a continuous line of WI-38 cells have been maintained in tissue culture for a minimum of 60 days. Aliquots of the cultured cells have been inoculated subcutaneously into newborn homologous animals as well as into Syrian hamsters and BALB/c mice. These animals are being maintained and monitored for tumors and/or other pathogenic responses. Currently, the smaller animals (Syrian hamsters and BALB/c mice) are being sacrificed according to a predetermined schedule and subjected to histopathological examination.

Other aliquots of the cultured cells have been seeded onto primary monolayers of human embryonic kidney, African green monkey kidney, duck embryo, rabbit kidney, mouse embryo and upon WI-38 cells. These feeder layers were observed for a minimum of 21 days for cytopathic or other effects. Agents thus revealed are being passaged to high titer and serologically classified and, when indicated, will be inoculated into newborn homologous and heterologous animals.

Two tissues from African green monkey kidney donors and canine kidney donors (laryngial and urethral), as well as all primary cell monolayers, are being screened for the presence of Mycoplasma. This screening utilizes two incubation conditions (aerobic and anerobic) and two different mediums. Mycoplasma revealed in this procedure are prepared at high titer and forwarded to another contractor (Huntingdon Research Center, Inc. #NIH-69-59) for serological identification. To date, 45 isolations have been accomplished as follows: canine tissues, 20; African green monkey tissues, 13; canine kidney cultures, 2; duck cells, 5 and WI-38 cells, 5.

Major Findings: In addition to the Mycoplasma isolations detailed above, it is apparent that the substrates examined in this contract also harbor

numerous covert viral agents. While insufficient time has elapsed for final conclusions to be drawn concerning oncogenic potential of the inoculated cells and adventitious agents, early observations appear to indicate no such potential in the smaller heterologous animals.

Significance to DBS Program and Bio-Medical Research: This is one of a number of experimental approaches designed to determine the presence or absence of covert oncogenic and/or other adventitious agents in cells used for vaccine virus production or in cells proposed for use in vaccine virus production. Results of this and related studies should allow the Division to evaluate the relative hazards of vaccine substrates and to make recommendations relating to their use.

Proposed Course of Project: This contract, which is scheduled for a two year period, began May 24, 1967, and is scheduled for termination May 23, 1969. Consideration is being given to the extension of this contract for a one year period.

Date Contract Initiated: May 24, 1967.

Current Annual Level: $117,795

CONTRACT NARRATIVE
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

I.I.T. RESEARCH INSTITUTE (PH43-67-1101)

Title: Chemical and Physical Analysis of Oils and Emulsifiers

Contractors' Project Director: Dr. Hugh J. O'Neill

Project Officer (DBS): Dr. Pinya Cohen, LBBP

Assistant Project Officer: Dr. M. Carolyn Hardegree, LBP

Objectives: Isolate and identify the major and minor components in Drakeol 6-VR mineral oil, peanut oil, aluminum monostearate and Arlacel A emulsifier.

Methods Employed: Detailed compositional analyses are performed chrough the combined use of column, thin-layer and gas chromatography; nuclear magnetic resonance; and infrared and mass spectrometry.

Major Findings: Both lots of mineral oil contain approximately 165 - 260 parts per million of monobutylphthalate and dibutylphthalate, respectively. This class of compounds is associated with plasticizers, insecticides and preservatives, however, the source of contamination has not been identified. These compounds are mildly toxic and have produced granulomas in rodents.

No polynuclear hydrocarbons have been found in mineral oil.

Mannide monooleate constitutes only 16 to 28% of two lots of Arlacel A analyzed. Older lots of Arlacel A show increased amounts of cyclic fatty acids and other U.V. absorbing components and polymeric carbohydrates. The cyclic components are toxic when placed in the diets of weanling rats. Fractions of Arlacel A emulsifier and mineral oil have been prepared for the Division.

The analysis of aluminum monostearate and peanut oil will be completed during the current year.

Significance to DBS Program and Bio-Medical Research: The procedures developed and analytical findings will be used to develop new criteria and specifications for defining the composition and safety of mineral and peanut oil; aluminum monostearate and mannide monooleate emulsifier which are components of experimental adjuvants.

Proposed Course of Project: Contract will terminate June 22, 1969.

Date Contract Initiated: May 23, 1967

Current Annual Level: $91,842

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

MELPAR, INC. PH43-67-1105)

Title: Electron Microscope Services

Contractor's Project Director: Robert Hermann

Project Officer (DBS): Ruth Dunlap, Ph. D.

Objectives: To utilize the electron microscope in the studies of vaccinia and other viruses.

Methods Employed: Sophisticated use of the electron microscope.

Major Findings: The electron microscope facilities at Melpar were used primarily in making vaccinia virus particle counts. This data was incorporated into a variety of studies described in Individual Project Report #124. Preliminary work of a similar nature was done to assess the virus content of influenza vaccines.

Significance to Bio-Medical Research and the Program of DBS: Electron microscopic examination of viral vaccines provides a quick method of determining the actual virus particle content. Since infectivity titrations vary from one host system to another, the particle count provides a base of reference to correlate titrations. It is also possible to assess the amount and character of debris present in the specimen, and the degree of aggregation of the virus particles. Information derived from electron microscopic examination is of great value in any type of quantitative work with viruses.

Proposed Course of Project: Contract was terminated May 21, 1969, as electron microscope services are now supplied to the investigator by the Laboratory of Biophysics and Biochemistry, DBS.

Date Contract Initiated: Contract was initiated May 22, 1967 for $14,560. It was renewed for 1 year as of May 22, 1968, at no additional funding.

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

IIT RESEARCH INSTITUTE (#PH-43-67-1106)

Title: Studies of Latent Infection and Tissue Culture Phase

Contractor's Project Director: Dr. H. M. Yamashiroya

Project Officers (DBS): Dr. B. G. Young (Latent Virus) and Dr. M. F. Barile (Mycoplasma)

Objectives: To investigate, by tissue culture methods, primary canine kidney, African green monkey kidney, bovine kidney, rabbit kidney, duck embryo, chick embryo and a continuous line of human lung fibroblasts (WI-38 cells) for covert contaminating viruses and Mycoplasma.

Methods Employed: Primary cultures of the above cells and continuous lines of WI-38 cells are being maintained in tissue culture for a minimum of 60 days. Aliquots of the cultured cells and aliquots of the cells disrupted by freezing and thawing are subjected to centrifugation procedures designed to concentrate any virus present. The final centrifugation is into agar pellets which are inoculated onto primary monolayers of human embryonic kidney, canine kidney, African green monkey kidney, duck embryo and onto WI-38 cells. These inoculated monolayers are further passaged for 15 passages and observed microscopically for cytopathic or other viral effects. Agents revealed by this procedure are being passaged to high titer, serologically classified, and subjected to neutralization attempts with the donor animals serum in order to demonstrate initial presence. When indicated, these agents will be provided to an animal phase contractor for *in vivo* studies.

Major Findings: Preliminary results indicate that a large number of cytopathic viral agents are being revealed by the technical approaches employed. Mycoplasma also is being isolated from each cell species tested. To date 9 isolations from canine tissue, 4 from African green monkey tissue, 3 from canine kidney cells, 2 from duck embryo and 3 from WI-38 cells have been accomplished. Considerable information has also been accumulated with respect to the incidence of intestinal and blood parasites infecting the donor animals.

Significance to DBS Program and Bio-Medical Research: This is one of a series of approaches designed to determine the presence or absence of covert viral agents and of Mycoplasma in cells used for vaccine virus production or in cells proposed for use in vaccine virus production. Results of this and related studies should allow the Division to make recommendations relating to the use of cell substrates for vaccine production.

Proposed Course of Project: This contract is currently scheduled for a fourteen-month period which began May 23, 1968, and is scheduled for termination July 22, 1969. The work was initiated May 23, 1967. Consideration is being given to the extension of this contract to allow study, in a similar fashion, of other tissues used or proposed for use as vaccine sutstrates.

Date Contract Initiated: May 23, 1967.

Current Annual Level: $100,244 for 14 months.

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

WOODARD RESEARCH CORPORATION (#PH-43-67-1397)

Title: Investigation of the Reaction Products of β-Propiolactone

Contractor's Project Director: Dr. G. Woodard

Project Officer (DBS): Dr. B. G. Young

Objectives: The reaction of β-propiolactone with a variety of biological materials is being investigated to determine the nature of the reaction products and any affinity of a particular substrate to β-propiolactone. The biological properties of β-propiolactone and its reaction products are also being determined by subcutaneous injection into weanling and newborn CAF_1 mice.

Methods Employed: Reaction products of β-propiolactone and biological materials separated and characterized by chromatographic procedures which employ ninhydrin and u.v. visualization to demonstrate their locations. Results are confirmed and extended employing β-propiolactone-H^3 and radioautography. Weanling and newborn CAF_1 mice have been injected subcutaneously with β-propiolactone and with the reaction products of β-propiolactone and ten selected biological materials to determine the presence or absence of oncogenic properties.

Major Findings: To date R_f values have been determined for all 36 biological test compounds. Reaction products have been detected in most instances. All mouse inoculations have been completed and the mice monitored for tumor production. A sacrifice schedule has been initiated and histopathological examination is nearing completion.

Significance to DBS Program and Bio-Medical Research: β-propiolactone is used as a virus inactivating agent in vaccine production. Non-hydrolyzed β-propiolactone has been reported to be mutagenic in Neurospora and oncogenic when applied to the skin of mice. During virus inactivation β-propiolactone is reported to be completely hydrolyzed to innocuous end products. Work conducted under this contract should demonstrate whether, during hydrolysis, β-propiolactone can impart oncogenic properties to non-oncogenic biological compounds. This knowledge will allow the Division to make recommendations on the continued use of β-propiolactone as a virus inactivating agent.

Proposed Course of Project: Work under this contract commenced June 28, 1967, and is expected to be completed June 27, 1969.

Date Contract Initiated: June 28, 1967

Current Annual Level: $49,645

YALE UNIVERSITY (#PH-43-67-1463)

Title: A Statistical Approach to Human Karyotypy.

Contractor's Project Director: Dr. Frank H. Ruddle

Project Officer (DBS): Dr. B. G. Young

Objectives: The purposes of this contract are to determine the precise definition of the chromosome constitution of man and to seek the solution of certain problems relating to the stability of cultured human cells.

Methods Employed: Lymphocyte cultures have been established from the cord blood of approximately 4500 human newborns and approximately 1000 of their mothers. Slides of metaphase chromosomes were prepared from these cultures and photographed. Idiograms were made from the photomicrographs and the chromosome arm length measurements were determined. Semi-automated scanning devices are also extensively used to supplement the conventional manual measurements. Primary diploid human cells in tissue culture (WI-38 cells) are also being examined to determine a correlation, if any, between karyotype and growth kinetics, mean cell volume, and ageing of the cells. Clinical coding of the data has been designed to permit direct punching of the data onto IBM cards. This data is then processed by computer.

Major Findings: To date all 4500 infant and 1000 mothers lymphocyte cultures have been initiated. Conventional karyotypes have been prepared and analyzed from 2500 of the infants and 250 mothers. An additional 2000 cells (from 1000 infants) have been photographed but not, as yet, karyotyped. Collection of background data on the newborns and their families has been completed. Data from 2500 have been processed through IBM cards and recorded on tape for analysis. Results to date indicate 0.5% (18 of 4000) of the chromosome complements are grossly abnormal including: translocations - 4; trisomy D- 1; trisomy E - 1; trisomy G - 3; XYY - 2; XXY - 3; XXX - 3; XO - 1. Only five of these infants were phenotypically abnormal. Approximately 25% of all infant karyotypes have demonstrated less drastic deviations (minor variants).

Results obtained to date with the human lung fibroblastic line (WI-38 cells) indicate that these cells have a finite life expectancy of about 50 cell doublings. With increasing passage number, the instantaneous growth rate constant decreases and is accompanied by a shortened lag period with an entrance into plateau phase at lower cell densities. A constant increase in mean cell volume occurs accompanied by an increase in numbers of cells with higher ploidy values. Total aneuploidy ranges from 3 - 5.5%. There is no clear cut trend in the level of aneuploidy over the finite life expectancy of the cell population. Two variant

subpopulations were detected which persisted for numerous passages but did not increase in the population.

Significance to DBS Program and Bio-Medical Research: This contract has two primary objectives. The first objective is to establish a reliable definition of the chromosome constitution of man and the second is the solution of problems relating to stability of continuous diploid human cell lines *in vitro* and the karyotypic response of these cell lines to infection with selected adventitious agents.

A precise definition of the chromosome constitution of man and a better understanding of the events associated with senescence in cultured human cells will allow the Division to make recommendations concerning the use of cultured human cells in vaccine production and may lead to new tissue culture techniques for evaluating the safety of biologics.

Proposed Course of Project: Work under this contract was initiated June 29, 1967. The contract has recently been renewed and it is anticipated that this project will be completed June 30, 1970.

Date Contract Initiated: June 29, 1967.

Current Annual Level: $241,475

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

BIONETICS RESEARCH LABORATORIES (PH-43-67-1483)

Title: Studies of the Oncogenic Activity of Human Biological Products in Syrian Golden Hamsters (Mesocricetus auratus)

Contractor's Project Director: Dr. John Landon

Project Officer (DBS): Dr. Stanley H. Singer

Objectives: To study human biological products for oncogenic activity when inoculated into newborn Syrian golden hamsters.

Methods Employed: The products to be studied are purchased from commercial sources in their final containers. These compounds are coded by DBS staff members and delivered to the contractor where they are inoculated into newborn hamsters within 24 hours after birth by a single subcutaneous inoculation. These animals are observed daily, weaned at 21 days, divided into groups of eight or less animals and observed closely for the development of palpable masses or other indications of neoplastic disease. All animals which die are subjected to thorough histopathologic examination except when cannibalism or autolysis will not permit. Transplants to weanling hamsters are attempted on all test animals which develop palpable masses. Blood serum and portions of the neoplasm are retained at -70°C. for further studies. Positive and negative controls are included in the study.

Major Findings: Animals inoculated with positive control materials have developed neoplasms after the proper latent period. There has been a higher than expected incidence of tumor formation in the remaining test groups, but because of the random distribution of these they are thought to reflect spontaneous tumor formation in the colony. Data received to date indicates no unusually high incidence of neoplasia in any of the test groups with the exception of the positive controls.

Significance to DBS Program and Bio-Medical Research: This is one approach to determine the safety of available human biological products. Although oncogenic activity in hamsters cannot presently be related to humans, results from hamster studies should shed some light on safety of these products.

Proposed Course of Project: This project is scheduled for termination in fiscal year 1970.

Date Contract Initiated: July 1, 1966

Current Annual Level: $168,031

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

Allergenic Products Section

UNIVERSITY OF CALIFORNIA MEDICAL SCHOOL (PH-43-68-632)

Title: Determination of Potency and Efficacy of the Active Ingredient of Poison Oak Extract by Clinical Studies

Contractor's Project Director: William B. Epstein, M. D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Henry Godfrey, M. D.

Objectives: To determine whether a pure separation of the active principle of poison oak can hyposensitize individuals sensitive to poison oak.

Methods Employed: The active principle was isolated from fresh leaves under Contract (PH-43-64-76) at Columbia University and was prepared for clinical use by the Pharmaceutical Development Service of the NIH. The contractor will administer this material to sensitive patients orally and parenterally. Skin tests prior to and during treatment will be used to assess efficacy. Blood and urine samples will be taken at regular intervals for laboratory examination to aid in determining whether the treatment produces any toxic effects.

Major Findings: Among the first group of sensitive individuals treated, there was a substantial reduction in skin-test reactivity.

Significance to DBS and Bio-Medical Research: It has been often stated, but not scientifically established, that the administration of poison oak extracts may reduce or eliminate the sensitivity of sensitive individuals. Since Poison Oak Extracts are subject to license, it would be of value to DBS to determine if they possess efficacy. If efficacy is established, it may be possible to establish useful dosage schedules, dosage sizes and the duration of the effect. It may also be possible to obtain information relating to the mechanism thus achieving a greater understanding of delayed contact sensitivity.

Proposed Course of Project: (1) To continue the study until it can be determined with certainty that desensitization does occur. (2) To ascertain a suitable dose and schedule of administration. (3) To determine the duration of desensitization after treatment is discontinued.

Date Contract Initiated: January 15, 1968

Current Annual Level: $10,800

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

UNIVERSITY OF MARYLAND (PH-43-68-646)

Title: Study of Recipients of Attenuated Measles Virus Vaccine Prepared in Canine Kidney Cells

Contractor's Project Officer: Dr. Fred R. McCrumb, Jr.

Project Officer (DBS): Dr. Stanley H. Singer

Objectives: This study is reviewing the health status of selected recipients of attenuated measles-virus vaccine.

Methods Employed: Data on health status is being collected by direct contact with physicians and household heads.

Major Findings: Preliminary data indicate no unexpectedly high levels of disease entities in the population under study.

Significance to DBS Program and Bio-Medical Research: This study is part of the continuing effort of monitoring the safety of vaccines.

Proposed Course of Project: This contract is anticipated to be completed by May 31, 1969.

Date Contract Initiated: February 28, 1969

Current Annual Level: $33,000

CONTRACT NARRATIVE
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

BIONETICS RESEARCH LABORATORIES, INC. (Ph 43-68-662)

Title: Housing and Maintenance of Rhesus Monkeys

Contractor's Project Director: David Valerio, D.V.M.

Project Officer (DBS): Nicola M. Tauraso, M.D.

Assistant Project Officer (DBS): Frank A. Pedreira, M.D.

Assistant Project Officer for Administration (DBS): Mr. Damian Crane

Objectives: The housing and care of rhesus monkeys.

Methods Employed: Animal quarters and routine animal care for 150 monkeys.

Major Findings: The following services were performed: (1) Daily boarding and maintenance of 150 monkeys; (2) Weekly blood samples collected from a group of 25 selected monkeys to be used for hematologic and chemical analyses; (3) Routine veterinary care and inoculation of animals; (4) Maintenance of health records on all animals; (5) Routine supervision and observation of animals.

Significance to Bio-Medical Research and the Program of DBS: Baseline hematologic values for normal rhesus monkeys are not readily available. However, baselines are useful in: (1) assessing the validity of an experimental value; (2) explaining fluctuations in data obtained over a period of time; (3) aiding in the choice of an animal for experimental studies; and (4) evaluating the effect of outside influences on the animal population. It is well known that variation is a common feature among animals living in the "wild." It is desirable to establish a baseline under controlled conditions for animals commonly used experimentally.

Proposed Course of Project: This project was terminated January 31, 1969.

Date Contract Initiated: Contract was initiated on June 28, 1968 for $35,225.

Current Annual Level: Amount expended as of January 26, 1969: $19,664.00.

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

Allergenic Products Section

PENNSYLVANIA STATE UNIVERSITY (PH-43-68-1250)

Title: Study of the Biochemical Properties of pollens and pollen extracts

Contractor's Project Director: Allen W. Benton, Ph. D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Henry P. Godfrey, M. D.

Objectives: (1) To carry out various physical and biochemical analyses of pollen extracts, including tests for enzymes. (2) To study the effects of various extracting fluids and methods and time of storage on the various components of the pollen extracts. (3) The effect of viability of the pollen and its age on the components of pollen extracts.

Methods Employed: (1) Pollens will be purchased at appropriate season of the year and extracts prepared using various extracting fluids including those commonly employed for preparing extracts. (2) Extracts will be studied for total protein content and for the presence of various enzymes mainly by disc electrophoresis and selective staining. Changes in composition of the extracts over various time intervals will be examined.

Major Findings: (1) Up to 10 protein bands have been found in ragweed pollen extracts. The extracts have been shown to contain several enzymes including acid and alkaline phosphatase. (2) Of the 7 extracting fluids used, all did not give equivalent extracts. (3) There appeared to be a loss of bands with storage.

Significance to DBS and Bio-Medical Research: Pollen extracts are products subject to license. Although it is known that these extracts contain many components including proteins, none has been identified by its biochemical activity. This project will provide a relatively simple method for identifying the proteins and ascribing enzymatic activity to certain of them. It will help to define the nature of changes that occur in extracts during storage.

Proposed Course of Project: To continue the study until the objectives are reached.

Date Contract Initiated: June 28, 1968

Current Annual Level: $22,245

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

Allergenic Products Section

OHIO STATE UNIVERSITY (NIH-69-65)

Title: Identification, isolation, and culture of mites from house dust

Contractor's Project Director: G. W. Wharton, Ph. D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): E. B. Seligmann Jr., Ph. D.

Objectives: (1) To examine house dust samples from various parts of the United States for their content of mites and other insects. (2) To determine the genus and species of the mites. (3) To isolate each species of mite, to produce pure cultures of each, and grow gram quantities of each.

Methods Employed: Various procedures suitable for isolating and culturing these animals will be explored.

Major Findings: (1) Mites of several known and unknown species have been found in the samples of house dust submitted by DBS to the contractor. No other insects have been recovered regularly. (2) Mites of at least two species are being grown in cultures.

Significance to DBS and Bio-Medical Research: It has been suggested that the allergenic activity of dust is due to its content of mites. Therefore the finding of mites in these dust samples is consistent with the concept. Further work will be required to determine whether or not mites in house dust can account for the allergy of individuals to dust and their skin reaction to house dust allergenic extract.

Proposed Course of Project: To continue until objectives are reached.

Date Contract Initiated: November 26, 1968

Current Annual Level: $30,000

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1969

Section on Viral Genetics

UNIVERSITY OF CHICAGO (NIH-69-85)

Title: True Incidence of Infectious Mononucleosis in Students

Contractor's Project Director: Dr. Richard A. Moy

Project Officer (DBS): Dr. Paul Gerber

Objectives: Sequential serum samples of students who were free of detectable EBV antibodies at the start of the academic year are examined for the appearance of such antibodies by complement-fixation tests. These findings are correlated with clinical and hematological data.

Major Findings: Study is still in progress, results are incomplete.

Significance to DBS Program and Bio-Medical Research: Obtain further sero-epidemiological evidence regarding the possible etiologic relationship of EB virus to infectious mononucleosis.

Proposed Course of Project: The current contract expires August 31, 1969 and the project will be reviewed at the completion of the current studies.

Date Contract Initiated: January 13, 1969

Current Annual Level: $6,048.

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

ELI LILLY LABORATORIES (NIH-69-98)

Title: Development of Sub-human Primate Diploid Cell Strains and Established Cell Lines

Contractor's Project Director: Dr. Robert N. Hull

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To develop diploid cell strains from immature male tissues of both the Macaca mulatta and Cercopithecus aethiops species of monkey which will be suitable for the production of viral vaccines for human use, and to compare the diploid cell strains with established cell lines derived from the same tissue.

Methods Employed: Tissues from selected immature male monkeys will be used to initiate cell cultures which will then be characterized with respect to growth, nutrient requirements, chromosome complement, oncogenic potential, viral susceptibility, and freedom from adventitious agents.

Major Findings: None to date.

Significance to DBS Program and Bio-Medical Research: The availability of a well-characterized cell system having genetic stability will allow exploration of the potential for vaccine production in cells other than primary cell cultures or human cell strains. In addition, the data obtained in the comparative study between diploid and aneuploid cells will be useful in assessing the significance of ploidy as a parameter in monitoring the safety of a cell culture.

Proposed Course of Project: This project is scheduled for termination in fiscal year 1971.

Date Contract Initiated: Approximately May 1, 1969

Current Annual Level: $131,462

CONTRACT NARRATIVES
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

LEDERLE LABORATORIES (NIH-69-100)

Title: Development of Sub-human Primate Diploid Cell Strains

Contractor's Project Director: Dr. Paul Vasington

Project Officer (DBS): Dr. John C. Petricciani

Objectives: To develop diploid cell strains from fetal tissues of both the Macaca mulatta and Cercopithecus aethiops species of monkey which will be suitable for the production of viral vaccines for human use.

Methods Employed: Fetal tissues from selected pregnant monkeys will be used to initiate cell cultures which will then be characterized with respect to growth, nutrient requirements, chromosome complement, oncogenic potential, viral susceptibility, and freedom from adventitious agents.

Major Findings: None to date.

Significance to DBS Program and Bio-Medical Research: The availability of a well-characterized cell system having genetic stability will allow exploration of the potential for vaccine production in cells other than primary cell cultures or human cell strains.

Proposed Course of Project: This project is scheduled for termination in fiscal year 1971.

Date Contract Initiated: Approximately May 1, 1969

Current Annual Level: $112,000

CONTRACT NARRATIVES
LABORATORY OF BACTERIAL PRODUCTS
DIVISION OF BIOLOGICS STANDARDS
July 1, 1968 through June 30, 1969

Allergenic Products Section

JOHNS HOPKINS UNIVERSITY (NIH-69-2010)

Title: Development of Information on the Activity of Allergenic Extracts

Contractor's Project Director: Philip Norman, M. D.

Project Officer (DBS): Harold Baer, Ph. D.

Assistant Project Officer (DBS): Henry Godfrey, M. D.

Objectives: To determine the human potency of allergenic extracts of grass pollens and giant ragweed.

Methods Employed: Test will be done in the skin of sensitive humans and using leucocytes of sensitive humans in the histamine release test.

Major Findings: The project has not yet started.

Significance to DBS and Bio-Medical Research: This project will attempt to correlate the human potency with a laboratory potency assay for allergenic extracts.

Proposed Course of Project: To continue until the objectives are attained.

Date Contract Initiated: March 1969.

Current Annual Level: $6320

CONTRACT NARRATIVE
LABORATORY OF VIRAL IMMUNOLOGY
Fiscal Year 1969

MELPAR, INC. (NIH-69-2013)

Title: Electron Microscopy Services

Contractor's Project Director: John Verna, Ph.D.

Project Officers (DBS): Lewellys F. Barker, M.D., LVI
David P. Stevens, M.D., LVI

Objectives: To perform electron microscopic examinations of thin section preparations of approximately 30 lymphoblastoid cell lines derived from the peripheral blood of patients with infectious hepatitis and other viral infections. An intensive examination to detect herpes-like viruses and any other viruses or virus-like particles is desired.

Methods Employed: Thin sections (0.1 μ) cut through cell nuclei will be selected for examination.

Major Findings: This project was recently initiated; no data yet available.

Significance to DBS Program and Bio-Medical Research: This contract provides service for one phase of a major hepatitis project; the dilution and identification of agents which are associated with hepatitis is extremely important.

Proposed Course of Project: The results obtained with the 30 cell lines will determined the future course of this approach.

Date Contract Initiated: March 26, 1969

Current Annual Level: $9,200.

Adjuncts to OD

ADJUNCTS

To The Office Of The Director

Serial No. DBS 01
1. Experimental Virology
2. Office of the Director
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Experimental Virology

Previous Serial Number: Same

Principal Investigator: Bernice E. Eddy, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description:

Objectives: To investigate the safety of biological products especially in relation to the oncogenicity of viruses and means of preventing neoplastic development; investigate new viruses and other agents in relation to their importance in human disease and for the purpose of exploring the possibility of preventing diseases that at present are uncontrolled.

Methods Employed: Conventional methods of propagating viruses in cell cultures and in animals, serological and cellular determination of antigenic relationships and pathological examination of tissues.

Major Findings: A continuing investigation in this section relates to the question of viral oncogenesis, and to the circumstances under which neoplastic changes in cells can be enhanced or supressed by an oncogenic virus or by antibodies induced by virus-induced neoplastic cells.

A non oncogenic or almost non oncogenic variant of adenovirus type 12 has been obtained by variation of the propagation procedure. Preliminary experiments have indicated that this variant is capable of protecting hamsters against tumors induced by the oncogenic parent strain.

Similar attempts have been made to obtain a nononcogenic simian virus 40 but to date only partial success has been achieved.

A tumor which arose in a hamster some months after injection with adenovirus type 4 is under investigation to determine if the tumor is related to the inoculum. Type 4 adenovirus is a common infection in man and has generally been regarded as a non oncogenic adenovirus.

A virus recovered after cultivation of a human carcinoma is also under investigation. This virus induces foci in a line of continuous cell cultures of human origin.

Simian virus 40-induced tumor cells which have been transplanted over 200 times in adult hamsters have been found to yield complete infectious virions after continued cultivation in vitro. These experiments lend credence to the idea that the causative virus in certain human tumors, in case one exists, can be propagated and identified.

With C. P. Li continued studies on the antitumor effect of clam extracts on virus induced hamster tumors.

Significance to Biomedical Research and the program of the Division: This program is of major significance in developing an understanding of hazards that may exist in proposed biological products and in providing information on the importance of the relationship of immunity and oncogenesis.

Proposed Course of Project: Work will be continued on immunologic effects in relation to oncogenicity of various viruses, employing techniques and experimental protocols similar to those above but varied in different parameters.

Publications:

Li, C. P., Prescott, B., Eddy, B. E., Chu, E. W. and Martino, E. C. Studies on inhibition of viral oncogenesis. I. Reduced tumor incidence in hamsters inoculated with adenovirus 12 and treated with clam extracts. J. Nat. Cancer Inst. 41:1249-1253, November 1968.

Eddy, B. E. A study of factors involved in oncogenesis in hamsters due to adenovirus type 12 or simian virus 40 (SV40). II Congreso Centroamericano y I Nacional de Microbiologic Panama, R. P. 2:52 December 1968.

Eddy, B. E. Monograph: Polyoma virus. Springer-Verlag, Inc. Wein and New York. In press.

Eddy, B. E. Infection with oncogenic viruses in Infectious Agents and Host Reactions (Stuart Mudd ed.) W. B. Saunders Co. Philadelphia. In press.

Annual Report of the Biometrics Section
Division of Biologics Standards
July 1, 1968 through June 30, 1969

I MISSION

The primary responsibility of the Biometrics Section, Division of Biologics Standards, is to insure that efficient statistical techniques are available and used in the scientific operations of the Division. For the most part, adequate techniques are available in the literature, but on occasion this will not be so. Even where appropriate techniques have been discussed, the literature may be scattered, highly technical, and contradictory, requiring study and comparison for adequate exploitation.

II ANALYSES SUMMARY

In Fiscal Year 1969, 50 specific tasks were initiated in the Section and 11 were continued from the previous fiscal year. Of these, 52 were completed, 3 were cancelled or suspended, and 6 are continuing.

III METHODOLOGICAL RESEARCH

1. A comprehensive review of the performance of the official potency test for tuberculin, in collaboration with other units of the Division, has been under way for some time. Completion of this study has been in suspense but should be resumed shortly. Evaluation of the tine-test with purified protein derivative for tuberculosis was carried out using a field trial involving some 18,000 widely distributed examinees. The data was supplied by the manufacturer in machine readable form. Several anomolous results were uncovered which still await resolution. A similar study is in progress using results of a manufacturer's field trial of comparable size but with old tuberculin as test material.

2. Research leading to an improved skin test for allergy is in progress in the Section on Allergenic Products. Data from a collaborative study with practicing allergists is being studied statistically to sharpen the results. Results as obtained are reported orally to the collaborators.

3. A new mathematical model developed to explain observed quantitative heterologous delayed sensitivity reaction among six atypical tuberculins studied in the Section on Mycobacterial and Fungal antigens is now being applied to eleven other atypical tuberculins. While study is just beginning, the prospects appear favorable that the model will fit satisfactorily. It is hoped that it may prove to be a useful tool in analyzing taxonomic relations as well as in the development of more specific tests for tuberculosis.

4. Choice between alternative measuring instruments or assessment procedures are based, among other things, on the precisions of determinations. Where this comparison is carried out as a supplement

to on-going application to real data, allowance must be made for variation from trial to trial of the quantity measured. A procedure for doing this was developed at Aberdeen Proving Ground in World War II but a satisfactory statistical test for the reality of the observed differences in test performance was not then available. In connection with a comparison of two methods of tetanus anti-titer assessment in the Laboratory of Bacterial Products, the need for such a statistical test was encountered. This test has now been developed and a manuscript describing it submitted for publication.

5. "Staging" of disease severity is applied in several disease states, including cancer, as an aid in choosing therapy and for other reasons. A disease severity index (Maloney 1961) is being applied to about 900 cases of breast cancer in collaboration with Mr. George Kennedy, statistical consultant, Wilmington, Delaware. Results are not yet available.

IV OPERATIONAL PROBLEMS

Statistical treatment of experimental data is heavily dependent on computer processing. Computer processing is also required in much statistical research. But, particularly in a research environment, computer processing requires large amounts of programming. No programming service has been obtained from the Computer Division for the past two report periods on two sub-tasks arising out of the DBS activities: Multinomial Acceptance Sampling and Exact Probabilities for Small Samples in Contingency Tables. Progress on these problems cannot procede until some means of obtaining sophisticated programming assistance is available.

A remote computer input has been obtained and is being gradually incorporated into the operation of the Section. It, of course, increases rather than decreases the need for programming.

V FUTURE PLANS

The research activities grow out of the consulting and statistical processing of the Section which cannot be anticipated. The two research tasks mentioned in Section IV will be resumed if and when programming facilities become available. The four uncompleted sub-tasks discussed in Section III being essentially computer independent will be continued.

TABLE I

SUMMARY OF STATISTICAL ANALYSES FOR FISCAL YEAR 1969

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
110	July 1966	Cancelled	Katti's Exact Probabilities	Dr. Maloney
127	February 1967		Evaluation of Standard Tuberculin Test, Part III	Mr. Kolb
144	July 1967	July 1968	Tuberculin Regression Comparison	Dr. Baer
146*	July 1967	October 1967	Branched Chains	Dr. Baer
156	October 1967	July 1968	Multiple Immunogens	Dr. Chaparas
160	October 1967		Tuberculin Analysis	Mr. Kolb
165	December 1967		Tuberculin Analysis	Mr. Kolb
171	January 1968	January 1969	Tine Effectiveness	Dr. Baer
173	January 1968		Disease Severity Index	Dr. Maloney
174	January 1968		Exact 3X3 Contingency Table Probability	Dr. Maloney
175	March 1968	March 1968	PPD Resubmission	Dr. Baer
176	May 1968	May 1968	Test for Normal Distribution	Dr. Smith
177	June 1968	July 1968	Complement	Dr. Spector
178	June 1968	July 1968	Serum Stabilization	Dr. Spector
179	June 1968	July 1968	Correlation	Dr. Godfrey

*not included in Fiscal Year 1968 Report

TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
180	June 1968	Suspended	Observer Error	Dr. Godfrey
181	June 1968	July 1968	Geometric Means	Dr. Godfrey
182	May 1968	June 1968	Urush Comparison	Dr. Baer
183	July 1968	July 1968	Migration Indices	Dr. Godfrey
184	July 1968	July 1968	Confidence Limits	Drs. Barile & Hardegree
185	July 1968	August 1968	Antibody Reactivity	Dr. Chaparas
186	July 1968	May 1969	Cholera Vaccine	Dr. Feeley
187	July 1968	May 1969	Cholera Reference	Drs. Feeley & Seligmann
188	July 1968	September 1968	Clam Liver Extract	Dr. Li
189	July 1968	September 1968	Geometric Means Catechols	Dr. Baer
190	July 1968	September 1968	Hamster Tumor Incidence	Dr. Li
191	July 1968	September 1968	Sex Response Difference	Dr. Li
192	July 1968	May 1969	Probit Mantoux-Tine Induration (Lederle Laboratory)	Dr. Baer
193	July 1968	August 1968	Regression Coefficients	Dr. Godfrey
194	August 1968	February 1969	Oil Suppression	Dr. Baer
195	August 1968	May 1969	Cholera Standard Calibration	Dr. Seligmann & Mr. Green

TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
196	August 1968	May 1969	Cholera Standard Calibration	Dr. Feeley
197	August 1968	August 1968	Confidence Limits	Dr. Chaparas
198	September 1968	December 1968	Analysis of Variance	Dr. Hardegree
199	September 1968	September 1968	Skin & Histamine Release	Dr. Baer
200	October 1968	October 1968	IND-216, Within Patient Test Reproducibility	Dr. O'Malley
201	October 1968	Cancelled	Sex Factor Tumor Incidence	Dr. Li
202	October 1968	January 1969	Hamster Tumor Incidence, Part II	Dr. Li
203	October 1968	January 1969	Tetanus Booster Persistence	Drs. Barile & Hardegree
204	November 1968	November 1968	Chi-Square: Confidence Limits	Dr. Morris
205	November 1968	April 1969	Typhoid Reproducibility	Dr. Seligmann
206	November 1968	March 1969	Allergen Histamine Correlation	Dr. Baer
207	December 1968	December 1968	Effect of Ring Substitution	Dr. Baer
208	December 1968	May 1969	Grouped Disproportionate Analysis of Variance	Dr. Baer
209	December 1968	December 1968	Geometric Means-DNBC	Dr. Godfrey
210	January 1969	March 1969	Correlation Between HSV&HLV (Burkitt vs. Control)	Dr. Gerber
211	January 1969	January 1969	Geometric Means-DNFB	Dr. Godfrey

TABLE I - continued

SAN	DATE INITIATED	DATE COMPLETED	TITLE	RESEARCHER
212	January 1969	June 1969	Model Test	Dr. Chaparas
213	January 1969	June 1969	Tine-Mantoux Reaction (Histoplasman)	Dr. Baer
214	February 1969	June 1969	Typhoid Vaccines	Dr. Pittman
215	February 1969	June 1969	Wyeth Typhoid	Mr. Green
216	February 1969	February 1969	Means and Standard Deviation	Dr. Tauraso
217	February 1969	May 1969	Tuberculin Bioassay	Mr. Crossley
218	February 1969	April 1969	Inaba Immunization	Dr. Miller
219	February 1969	June 1969	Sample Size Requirement	Dr. Smith
220	March 1969	June 1969	Heterologous Standardization	Dr. Chaparas
221	March 1969	April 1969	Influenza Strains	Dr. Tauraso
222	March 1969	April 1969	Lot 6A	Dr. Seligmann
223	March 1969	April 1969	Confidence Limits	Dr. Seligmann
224	April 1969	April 1969	Y F Serum Titer	Dr. Tauraso
225	April 1969		Lupus Erythematosus	Dr. Maloney

Serial No. DBS 111
1. Office of the Director
2. Biometrics Section, DBS
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Development of Statistical Methodology Applicable to Biologics Control.

Previous Serial Number: DBS 111.

Principal Investigator: Clifford J. Maloney, Ph.D.

Other Investigators: Harold Baer, Ph.D.; S. D. Chaparas, Ph.D.

Cooperating Units: Section on Allergenic Products and Section on Mycobacterial and Fungal Antigens, DBS.

Man Years:

Total:	0.5
Professional:	0.3
Other:	0.2

Project Description:

Objective: As need arises from the research activity of the Division for statistical technique not adequately treated in the literature and when it appears that a need for such technique will continue on an appreciable scale in the future, theoretical and computational work is undertaken to develop appropriate technical tools.

Subproject 1: The field performance of the Tine test for tuberculosis antibody titer using OT is required to judge suitability for license.

Subproject 2: The logical structure of the quantitative relations between homologous and heterologous challenge for atypical mycobacteria is desired as an aid in purifying allergenic test fractions.

Subproject 3: A significance test to distinguish between measuring instruments and test procedures on the basis of precision when used on inherently variable material is required.

Subproject 4: The development of an improved method for disease staging would be helpful.

Methods:

Subproject 1: Contingency table analysis is being applied to some 15,000 examinations from a manufacturer supplied in machine readable form.

Subproject 2: A technique of ordering rows and columns of a matrix of observations subject to experiment error previously developed by the principle investigator was adapted to the analysis of results from heterologous delayed sensitivity skin tests.

Subproject 3: The likelihood ratio test was applied to a mathematical model describing the conditions of the required test.

Subproject 4: A probabilistic model of disease severity quantitation is being applied to some 900 cases of breast cancer.

Findings:

Subproject 1: None. Analysis is just under way.

Subproject 2: None. Analysis is just beginning.

Subproject 3: It was shown that the Pitman-Morgan test applied to this situation. The power of the test for a few cases were calculated. A paper describing the work has been prepared and is being offered for publication.

Subproject 4: None. Analysis is just beginning.

Significance to Bio-Medical Research and the Program of the Division:

The current studies (a) solve the problem of an exact test for equality of correlated variances, (b) may contribute to the elucidation of the antigenic and/or taxonomic relations of atypical tuberculins, (c) improve the staging of disease and (d) examine the suitability of OT tine test for tuberculin antibody titer.

Honors and Awards: None

Publications: None

Annual Report of the Office of Investigational New Drugs
Division of Biologics Standards
July 1, 1968 - June 30, 1969

This office has the continuing responsibility for the receipt and evaluation of "Notices of Claimed Investigational Exemption" for new biological products.

During the period covered by this report 66 "Notices" were received. This brought to 374 the total number of "Notices" received since the Food and Drug Regulations requiring submissions became effective. Disposition of "Notices" received during the period 1963-1969 is shown in the following table:

Active	239
Withdrawn	134
Forwarded to FDA for processing	1
Total	374

The number of supplements received on active INDs throughout the year depends on the activity and number of investigators involved in the respective clinical trials. Following initial review of the "Notices", this office has the responsibility for informing sponsors of deficiencies in the submissions. Liaison with the sponsors and the Food and Drug Administration is maintained.

The information contained in these "Notices" may indicate to the scientific staff of the Division the paths in which research should be directed for the purpose of developing Regulations for monitoring the safety and potency of new biological products. Sponsors of INDs are encouraged to submit all available data for review so that judgments as to the safety and potency can be made well in advance of the time of licensing.

The members of this office participate actively in the scientific life of the DBS and of the National Institutes of Health attending conferences and seminars as well as representing the Division in a liaison capacity with appropriate committees of other institutes. Conferences are held periodically with representatives of manufacturers and other interested in investigative new drugs.

Annual Report of the Contract Operations Office
Division of Biologics Standards
July 1, 1968 through June 30, 1969

In 1966, the Division established a contracts program to serve as an extension of, and a supplement to, its research and control activities. Among the principal questions to be investigated was the possible oncogenicity of viral vaccines and the carcinogenic or toxic properties of vaccine components, adjuvants, and other biologics. Since that time, approximately 40 contracts have been initiated to study oncogenicity and other questions pertinent to the Division's role as the regulatory body of the NIH. Many of these contracts are nearing completion and it is anticipated that much significant data should become available during the coming year.

The Division presently supports twenty-nine (29) active contracts. It is anticipated that the number of contracts will remain relatively constant as completed projects will be balanced by new project needs.

In those instances where Project Officers are assigned from the individual laboratories to manage the projects, details of each contract which they monitor will be found under a "Contract Narrative." Other projects, initiated and managed by the Contracts Staff of the Office of the Director, are also included in the special Index to Contracts in this report.

Although the contracts staff personnel participate extensively in the supervision of contract operations, providing materials for texts and examining results of day-to-day activities, the scientific staff of the Division also contributes greatly to the development and function of the contracts program. This contribution has been of particular value in the initiation, supervision and evaluation of the contract efforts. Often this has involved considerable expenditure of time, made at a sacrifice to their own programs. Members of the contract staff are also engaged in direct research.

Serial No. 131
1. Office of the Director
2. Contract Operations Office
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of Interferon Production

Previous Serial Number: 131

Principal Investigator: Stanley H. Singer, M.D.

Other Investigators: Ruth L. Kirschstein, M.D.
Michael Barile, Ph.D.
Margaret Shaffer, B.S.

Cooperating Units: Laboratory of Pathology, DBS
Laboratory of Bacterial Products, DBS

Man Years:
Total: 2.0
Professional: 1.0
Other: 1.0

Project Description:

Objectives: The effect of chronic infections with certain viruses or mycoplasma on subsequent virus-cell interactions, with particular emphasis on interferon production, is under study.

Methods Employed: Cultures are first inoculated with certain viruses or mycoplasma in order to initiate chronic infection. The cultures are then superinfected with other viruses whose adsorption, penetration, replication, and ability to induce interferon is evaluated.

Major Findings: The principal findings indicate that mycoplasma infected cells have a decreased ability to produce and react to interferon, an antiviral protein naturally produced during the course of viral infections. Viral transcription in these cells was not affected. The net result of these changes was that mycoplasma infected cells were able to yield significantly higher amounts of virus than non-mycoplasma infected cells. The presence of mycoplasma also decreased the ability of cells to respond to a synthetic interferon inducer, namely, polyinosinic acid:polycytidilic acid.

Significance to Bio-Medical Research and the Program of the Division: The results indicate that the presence of a common cell contaminant, mycoplasma, can significantly alter virus-cell interactions. Because mycoplasma

infected cells are capable of yielding greater amounts of virus than non-mycoplasma infected cells, it is possible that the course of naturally occurring viral infections in vivo may be altered by the presence of mycoplasma.

Proposed Course of Project: These studies will terminate at the end of this fiscal year when the principal investigator leaves to resume residency training.

Honors and Awards: None

Publications:

Singer, S. H., Kirschstein, R. L., and Baron, S., Viral Inhibition of Adenovirus 5 Treated Hamster Cells, Proc. Soc. Exp. Biol. and Med., 130:376-381, 1969.

Singer, Stanley H., Barile, Michael F., and Kirschstein, Ruth L., Enhanced Virus Yields and Decreased Interferon Production in Mycoplasma Infected Hamster Cells, Proc. Soc. Exp. Biol. and Med. (in press).

Singer, Stanley H., Kirschstein, Ruth L., and Barile, Michael F., Enhanced Vesicular Stomatitis Virus Yields from Mycoplasma Infected Hamster Cells, Nature (in press).

Serial No. DBS 132
1. Office of the Director
2. Contract Operations Office
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Studies of Virus-Cell Interactions

Previous Serial Number: DBS-132

Principal Investigator: B. G. Young, Ph.D.

Other Investigators: B. E. (Engels) Swart, B.A.

Cooperating Units: None

Man Years

Total:	1.70
Professional:	1.00
Other:	0.70

Project Description:

Objectives: To investigate the role of interferon in the maintenance of persistent virus infection of cultured human lymphoblasts derived from Burkitt's lymphoma and leukamias. Also, to study the physiological responses of these cell-virus systems to chemical inhibitors of DNA, RNA, protein and interferon synthesis. Special attention has been given to the immunologic character of the infected cell, the reactions of this cell with "normal" globulins of human origin and the interferon content of seven of these continuous cell lines. Correlations between virus content, positive indirect immunofluorescence, sensitivity to a cytotoxic reaction and interferon content of these cell lines have been established.

Methods Employed: Virus infectivity titrations in tissue culture, quantitation of interferon production and fluorescence microscopy are some of the methods employed to study these selected virus-cell systems.

Major Findings: Seven continuous cell strains, derived from human lymphomas and leukemias, have been characterized as to their latent virus content and interferon production. Without exception an inverse relationship has been demonstrated, i.e., the greater the severity of the virus infection the lesser the interferon production.

Actinomycin D specifically inhibits DNA-dependent RNA synthesis as well as the cellular production of interferon. Exposure to Actinomycin D causes the numbers of virus containing human lymphoma cells to increase

in culture 3-7 fold. This increase is followed by an increase in cellular interferon synthesis. Thus, a balance between latent intracellular virus content and cellular interferon synthesis has been demonstrated.

Normal pools of human immune globulins contain globulins which specifically react with virus containing human lymphoma or leukemia cells in vitro. This reaction results in suppression of growth and in the production of positive immunofluorescent reactions. The immunofluorescence titers and growth suppression titers of 20 normal pools of human immune globulins have been established. Good agreement was found for both titers indicating the probable identity of the globulins. The biophysical properties of the globulins were also studied.

Significance to Bio-Medical Research and the Program of the Division: The etiologic significance of the "herpes-like" virus ubiquitously present in cultured human lymphoma and leukemia cells has yet to be firmly established. The demonstration of the presence of growth suppressive antibody and the frequency of this occurrence in "normal" pools of human globulins should contribute to an assessment of the etiologic significance of the virus and may lead to the development of new therapeutic approaches to this unique malignancy. Similarly a determination of the quantity of interferon present in continuous lines, correlated with the presence or absence of virus, may also contribute to the evaluation of the etiologic significance of the virus and allow a better understanding of how persistent virus infections are maintained.

Proposed Course of Project: These studies will continue in the coming year.

Honors and Awards: None.

Publications: Swart, B. E., and Young, B. G.: Inverse Relationship of Interferon Production and Virus Content in Cell Lines from Burkitt's Lymphoma and Acute Leukemias. J. Natl. Cancer Inst. June, 1969.

Serial No. DBS 174
1. Contract Operations Office
2. Office of the Director
3. Bethesda, Md.

PHS-NIH
Individual Project Report
July 1, 1968 through June 30, 1969

Project Title: Investigation of Seven Compounds Commonly Used in Biological Products

Previous Serial Number: NONE

Principal Investigators: Edward A. Fitzgerald
Carl E. Miller, D.V.M.

Other Investigator: None

Cooperating Unit: None

Man Years

Total:	2.0
Professional:	1.0
Other	1.0

Project Description: Investigation of Compounds Used in Biological Products

Objectives: To determine if seven of the chemically defined constituents of biological products have any oncogenic properties or potential oncogenic properties.

Methods Employed: Seven chemicals, Benzethonium Chloride, Ethylene Chlorohydrin, Phenol Red, Ethylene Glycol, Parabens, Thimerosol, and Pyridine were selected for testing. A random bred Swiss strain of mice and an inbred strain of mice, Balb/c, were used in the study. Each strain was divided into 8 groups (including control) of 50 mice each, x 2 sexes, x 2 routes x 2 strains x 2 replicates for a total of 6400 mice. One half of the eight groups were to receive a single injection of the chemical and the other half received multiple injections. The routes were subcutaneous and intraperitoneal. The animals were observed daily and the sick animals removed, necropsied, and placed in formalin. After 18 months, all remaining animals in a cage were killed, examined for masses. The mice with abnormalities were necropsied plus enough others to include at least 5 mice from each group of 25.

Major Findings: All animals have been disposed of. Forty-six and eight tenths percent (3068) of the mice were necropsied and placed in

formalin. Twenty-one and nine tenths percent (1432) of the mice were palpated and discarded as having no unidentified masses. Approximately 15.6% of the animals died during the experimental period, and the same number were lost to the experiment through escapes in transit, earlier necropsies, etc.

The spontaneous lymphomas and unidentified masses were tabulated from the Swiss mice. No unusual increase in any of the groups over the control groups was evident from gross examination. In the Balb/c mice, lung tumors and unidentified masses were tabulated with no unusual increases noted in any of these mice after gross examination.

Significance to Bio-medical Research and Program of the Division: The reaction of animals to the pure chemicals used in various vaccines is one step in obtaining data to indicate the relative safety for use in humans. The more information that can be accumulated for a given compound, the more assurance one has for allowing the compound to be used in a lesser concentration in human vaccines. The information obtained from this project, coupled with the information from three contracts testing the same compounds will provide considerable data about the safety of these seven compounds.

Proposed Course of Project: Termination.

Honors and Awards: None

Publications: None

Annual Report of the Public Information Office
Division of Biologics Standards
July 1, 1968 - June 30, 1969

The responsibility of the DBS Public Information Office to create a better understanding by the public of the aims and accomplishments of the Division was carried out through various media:

PRESS: Hong Kong flu elicited a heavy volume of calls beginning in July 1968 and peaking in December. Wire services, news bureaus, news syndicates were updated daily, and detailed information on the DBS role was furnished U.S. and Canadian newspapers. National magazine features researched through information office: JAMA, Science, Time.

Rubella vaccine was second in press interest. National magazine features researched through this office, and photos supplied: Medical World News, May 16, '69, cover story; Today's Health, June '69, lead article; Reader's Digest, April '69, lead article; Hearing and Speech News, Nov.-Dec., 1968, cover story.

NEWS RELEASES: Joint release on International Rubella Immunization Conference, November 22, mailed out on DBS mailing keys to newspapers, journals and magazines throughout the country, brought heavy volume of queries. Press room coverage during 3-day conference included many requests for photos, background material on DBS, arrangements for staff interviews and distribution of papers presented by DBS staff.

Release on 1969 Max Weinstein Award presentation to two DBS scientists by United Cerebral Palsy Associations including photos mailed on request and assistance to UCPA PR staff.

Release on rubella vaccine Notice of Proposed Rule Making, Federal Register, April 3, 1969, (required by DHEW) resulted in heavy volume of queries from press, radio, and TV regarding impending licensing of vaccine.

Release on revised typhoid vaccine regulations, Federal Register, May, 1969.

RADIO AND TV: Coverage of Hong Kong flu included ½-hour live interview with Dr. Tauraso on CBS "Capitol Tieline," a 20-minute taped interview with Dr. Wagner on WMAL "University Comments," filmed interview in lab with Dr. Seligmann, ABC TV news. Recorded statements on flu by DBS staff were aired by Westinghouse Broadcasting Co., ABC radio (Chicago). Fifteen-minute interview on "Poison Ivy Sensitization," Dr. Baer, WGMS, April 25, '69. Voice of America: interview with Director, Nov. 20, '69; interview with Mrs. Meyer on rubella to French-speaking West Africa, April 25, '69.

DBS BROCHURE DISTRIBUTION: Volume of requests for brochure after initial distribution, plus distribution at FASEB, ASM, AABB meetings and Rubella Conference, has depleted stock to less than 2,000. Requests included foreign biological laboratories, WHO headquarters in Geneva, PAHO, as well as state health departments, hospitals, medical colleges, pharmaceutical manufacturers, voluntary organizations, government and independent research

organizations, and medical libraries. Reorder of brochure through GPO is planned.

"BLOOD AND THE RH FACTOR" REVISION: Continuing demand by DHEW Public Inquiries Branch required revision to include information on use of Rh immunoglobulin for prevention of Rh disease.

TEXT BOOK MATERIAL: Glossy prints and captions on DBS laboratory procedures furnished World Book Encyclopedia and two science textbooks. Material used to describe Division's function with respect to licensing and control of biologics.

FILMS AND PHOTOGRAPHY: Film footage on rubella, taken in DBS labs and in Conway, Arkansas (DBS rubella pilot studies) by NIAID and MS&D film crews has been scheduled to date for: 1) USIA's "Science Report of USIA"--15-minute film on rubella, for use abroad; 2) Network for Continuing Medical Education (sponsored as a public service by Hoffmann-La Roche through CDC)--closed circuit TV to hospitals, medical schools, societies, and clinics on use of vaccine and DBS research; 3) PHS film to motivate public participation in vaccination program; 4) Metromedia--three 5-minute programs on rubella vaccine at time of licensing; 5) ABC network 5-minute "cameo" spots on the vaccine licensing. Many other uses of the DBS footage are planned.

Demands for photographs of DBS staff and laboratory techniques were numerous in connection with Rubella Conference. Photo features include for overseas use by London science writer, and a 2-page spread of photos for NIH Record (April 15).

SPECIAL PROJECTS: 1) LBBP exhibit for AABB meeting. Mounted arrangement of photo enlargements of laboratory techniques attracted many visitors (Oct. 1968). 2) Wrote ten-page history of DBS during the Johnson Administration for inclusion in History of NIH for Johnson Library (July 1968). 3) Collected material (reprints, press releases, resumés of collaborative studies) on international activities of DBS for Fogarty International Center (April 1969). 4) Collaborated on answers to questionnaire for Senate Subcommittee on Administrative Practices and Procedures (March 1969). 5) Compiled a mailing key for distribution by NIH Printing and Reproduction of informational materials. Key includes 600 mailing addresses of science writers, newspapers, news services, TV and radio, magazines, affiliated national agencies, state medical societies, and government organizations.

RECURRING PROJECTS: DHEW Annual Report; NIH Brochure revision; NIH Directory-Bibliography; 1968 collection of DBS published papers for binding; NIH Almnac revision; DBS Who's Who; announcement and distribution of flyers for DBS research seminars; Special Report on Rubella (Congress); DBS Highlights (Congress); Report on Information Activities (Congress). Note: Due to work load and short staff, DBS Who's Who cannot be revised in 1969.

Annual Report of the Office of Scientific Communications
Division of Biologics Standards
July 1, 1968 through June 30, 1969

The responsibilities of the DBS Scientific and Technical Communications Office (OSC) are to plan, organize and implement appropriate programs for the collection, evaluation, storage, retrieval, and distribution of the scientific and technical data and information needed for the control and research programs of the Division.

The implementation, last year, of a rapid literature searching service (contract No. PH43-67-74) made it possible for the OSC to handle over 150 specific, and often complex, search requests and to offer the staff over 8,000 individual abstracts in response to specific technical problems. This program has been temporarily suspended due to shortage of personnel.

A selective bibliography, containing over 700 references covering the world's research on rubella was prepared for the Rubella Conference in February, 1969. The OSC also compiled a selective citation bibliography on anti-lymphocyte serum containing over 200 references.

The in depth indexing, of the World Health Organization working documents of the Expert Committee on Biological Standardization from 1947 to data, completed last year has been updated, and duplicates of the original master file of 10,000 cards was sent to the WHO headquarters in Geneva, Switzerland.

Automation and indexing of the Central Files, mentioned last year, has been postponed until 1970 due to lack of funds and personnel.

The DBS library and reference room was reorganized for more effective staff use.

The informal network for sources of information continues to grow each year and now includes not only other Divisions, Institutes, Federal agencies, private industries, societies and libraries but many individual scientists in the private and university sectors. Over 250 different individual sources for information were consulted during this fiscal year by the OSC to meet Division problems and needs.

Routinely the OSC assembles and indexes the Division's Annual Report each year.

The utilization of the most effective procedures for meeting the scientific and technical information needs of the Division continues to be the main objective of the office. However, limited funds and personnel make long-range planning difficult.

Contracts

INDEX TO CONTRACTS
Division of Biologics Standards
July 1, 1968 through June 30, 1969

Number	Subject	Page
PH-43-64-76	Poison Ivy	256
PH-43-64-885	Poison Ivy	258
PH-43-65-1046 PH-43-68-97)	Plastic Containers	259
PH-43-66-1163	Mycoplasma Antigens	261
PH-43-67-675	Chemical Carcinogenesis	262
PH-43-67-677 PH-43-67-676 PH-43-67-684	Carcinogenesis	264
PH-43-67-679	Oil Adjuvants	266
PH-43-67-701 PH-43-67-705	Exchange Transfusions	268
PH-43-67-1100	Latent Infections	270
PH-43-67-1101	Oils & Emulsifiers	272
PH-43-67-1105	Electron Microscope	274
PH-43-67-1106	Latent Infection	275
PH-43-67-1397	Beta-Propiolactone	277
PH-43-67-1463	Human Karyotypy	278
PH-43-67-1483	Oncogenic Activity	280
PH-43-68-632	Poison Oak	281
PH-43-68-646	Measles Vaccine	282
PH-43-68-662	Rhesus Monkeys	283
PH-43-68-1250	Pollen Extracts	284
NIH-69-65	House Dust	285
NIH-69-85	Infectious Mononucleosis	286

Number	Subject	Page
NIH-69-98	Diploid Cell Strains	287
NIH-69-100	Diploid Cell Strains	288
NIH-69-2010	Allergenic Extracts	289
NIH-69-2013	Electron Microscopy	290

Investigators

INDEX TO INVESTIGATORS
Division of Biologics Standards
July 1, 1968 through June 30, 1969

Abels, B. R. . . . p. 198

Albrecht, Paul. . . . p. 121, 122, 193, 195, 197, 198, 199

Allen, A. M. . . . p. 204

Aronson, David L. . . . . p. 70, 71, 73, 105

Auerbach, Robert. . . . . p. 258

Aulisio, Calvin G. . . . p. 204, 214, 217, 218, 219, 221

Aylor, Harry T. . . . p. 99

Axelrod, David. . . . p. 119

Baer, Harold. . . . p. 7, 9, 10, 11, 12, 13, 15, 17, 130, 133, 256, 258, 281, 284, 285, 289, 297, 298, 299, 300, 301 311

Baker, James. . . . p. 235

Barile, Michael F. . . . p. 18, 22, 24, 46, 47, 48, 50, 120, 128, 129, 250, 261, 270, 275, 298, 299, 305, 306

Baridon, Laura. . . . p. 171

Barker, Lewellys F. . . . p. 52, 56, 59, 130, 132, 159, 160, 165, 170, 243, 290

Baron, Samuel. . . . p. 117, 119, 129, 306

Bennett, John M. . . . p. 158

Benton, Allen W. . . . p. 101, 284

Berard, Costan W. . . . p. 158

Berkeley, William H. . . . p. 89

Berman, B. A. . . . p. 15

Biehusen, F. C. . . . p. 177

Blass, John P. . . . p. 161

Brady, Roscoe O., Jr. . . . p. 161

Branch, John. . . . p. 157, 158

Bridge, Allyn G. . . . p. 268

Brown, Eva R. . . . p. 146, 165

Brown, Paul. . . . p. 250

Butterly, Arlene. . . . p. 311, Introduction

Buynak, E. B. . . . p. 149

Canchola, Jose G. . . . p. 179, 209, Introduction

Carbone, Paul P. . . . p. 158

Carpenter, C. C. J. . . . p. 29

Carroll, William R. . . . p. 70

Carski, Theodore R. . . . p. 48. 250, 261

Chaparas, Sotiros D. . . . p. 7, 9, 10, 38, 39, 40, 41, 42, 45, 85, 117, 119, 121, 297, 298, 299, 300, 301

Chen, K. M. . . . p. 250

Chu, E. W. . . . p. 293

Clark, Glennelle D. . . . p. 52, 55, 58, 59

Clark, Ronald G. . . . p. 30, 45, 112, 115, 117, 121, 122, 123, 126, 193, Introduction

Coghill, G. E. . . . p. 213

Cohen, Martin H. . . . p. 158

Cohen, Pinya. . . . p. 67, 68, 262, 266, 272

Cooper, L. Z. . . . p. 151

Cook, R. O. . . . p. 201

Cox, Claire. . . . p. 113, Introduction

Crane, Damian. . . . p. 283, 304

Crossley, Ray D. . . . p. 300

Crouch, Madge L. . . . p. 259

Dawson, Charles R. . . . p. 256

Dawson, John H. . . . p. 2, 12, 89

Davis, William J. . . . p. 146, 156, 171, Introduction

DelGiudice, Richard A. . . . p. 48, 250

DeMeo, Anthony. . . . p. 42, 43

Diamond, L. S. . . . p. 40

Douglas, Robert D. . . . p. 146, 168, 170, 175, 177

Dowdle, Walter. . . . p. 251

Dunlap, Ruth C. . . . p. 52, 55, 179, 223, 274, Introduction

Dunn, Ellis. . . . 67, 68

Eddy, Bernice. . . . p. 251, 292, 293, 294, Introduction

Ehrenreich, B. Lynne. . . . p. 70, 71, 74

Engels, B. E. . . . p. 307

Ennis, F. A. . . . p. 177

Enterline, Joan C. . . . p. 146, 173

Epstein, William B. . . . p. 281

Espana, Carlos D. . . . p. 202

Esposito, Vito M. . . . p. 1, 26, 32, 61

Fales, H. M. . . . p. 164

Farber, Jane F. . . . p. 86, 91, 93, 97, 101, 103, 108, 207

Feeley, John C. . . . p. 7, 26, 29, 32, 34, 298

Fike, Colonel Robert . . . . p. 165

Finlayson, John. . . . p. 62, 70, 71, 73, 74, 75

Fitzgerald, Edward. . . . p. 113, 309, Introduction

Ford, Marshall. . . . p. 130

Francis, Thomas C. . . . p. 46, 47

Frantantoni, Joseph C. . . . p. 161, 164

Friedman, J.A. . . . p. 73

Fujimoto, Wilfred. . . . p. 161, 164

Fuller, Vernon. . . . p. 39, 85, 95, 107

Gajdusek, D. C. . . . p. 198, 250

Gallelli, Joseph. . . . p. 9

Gangarosa, E. J. . . . p. 4, 27

Garman, Robert. . . . p. 42, 43, 251

Gehle, Warren D. . . . p. 52, 53, 56, 58, 59, 165

Gerber, Paul. . . . p. 157, 158, 169, 286, 299

Gibbs, Clarence J. . . . p. 48, 198, 250

Gibson, Samuel T. . . . p. Introduction

Giovanni, Di Chiro. . . . p. 122

Godfrey, Henry P. . . . p. 9, 10, 11, 13, 256, 258, 281, 284, 289, 297, 298, 299

Grabowski, Marian W. . . . p. 48

Grater, William B. . . . p. 15

Graykowski, Edward A. . . . p. 46

Green, Otis L. . . . p. 99, 298, 300

Grubbs, Robert E. Jr. . . . p. 87

Guess, Wallace L. . . . p. 259

Hale, R. . . . p. 15

Hann, W. D. . . . p. 195

Hansen, Carl T. . . . p. 36

Hardegree, M. Carolyn. . . . p. 18, 20, 22, 24, 25, 117, 118, 119, 262, 266, 272, 298, 299

Hart, D. W. . . . p. 73

Harvey, Estelle. . . . p. 52, 53, 59

Hedrick, Sally R. . . . p. 38, 42, 45

Helprin, Jerome J. . . . p. Introduction

Hermann, Robert. . . . p. 274

Herndon, J. H. . . . p. 164

Hill, Donald E. . . . p. 13

Hilleman, M. R. . . . p. 149

Hinkle, William J. . . . p. 36

Hirschman, R. J. . . . p. 52, 56, 59, 165

Hobbins, Thomas E. . . . p. 52, 59, 146, 156, 173, 177

Hochstein, Donald H. . . . p. 26

Homburger, Freddy. . . . p. 264

Hopps, Hope E. . . . p. 123, 146, 155, 156, 159, 165, 168, 170, 175, 177, 261

Horton, A. Wesley. . . . p. 262

Hudson, Herman. . . . p. 67, 70, 71, 74, 75

Hull, Robert N. . . . p. 287

Jahnes, William G. . . . p. 205, 207, 214, 216, 219, 221

Jacobson, Cecil B. . . . p. 161, 164

Johnson, Joyce. . . . p. 121, 126

Judge, Frances. . . . p. 251

Kanfer, J. N. . . . p. 164

Kaufman, Seymour. . . . p. 161

Kies, Marian. . . . p. 45

Kingsburg, David T. . . . p. 229, 230

Kirschstein, Ruth. . . . p. 24, 25, 36, 41, 48, 49, 111, 113, 114, 115, 116, 117, 119, 120, 123, 125, 128, 129, 130, 135, 165, 193, 205, 207, 211, 305, 306, Introduction

Klutch, M. J. . . . p. 201, 213

Kolb, Robert W. . . . p. 22, 297

Kolodny, Edwin H. . . . p. 161

Krugman, Saul. . . . p. 151, Introduction

Kurtz, S. P. . . . p. 12

Lanchantin, G. F. . . . p. 73

Landon, John. . . . p. 280

Lapey, Allen. . . . p. 161

Larson, H. Elliott. . . . p. 146, 156

Lee, A. M. . . . p. 211

Leventhal , Brigid G. . . . p. 48

Levy, Hilton. . . . p. 117, 119

Li, C. P. . . . p. 179, 293, 298, 299, Introduction

Lichenstein, Lawrence. . . . p. 13

Lorenz, Douglas. . . . p. 17, 130, 165

Lunde, M. N. . . . p. 40

McCrumb, Fred R., Jr. . . . p. 282

McGrath, Philip. . . . p. 121, 122

Maloney, Clifford J. . . . p. 11, 15, 42, 295, 297, 300, 301

Manclark, Charles R. . . . p. 30, 36, 121, 122

Marquina, Ruben. . . . p. 85, 95, 107

Marshall, Harry. . . . p. 20, 83, 93

Marshall, James F. . . . p. 30, 36

Matters, B. A. . . . p. 151

Mason, Marcus. . . . p. 264

Mason, Richard A. . . . p. 225, 227, 229, 241

Martino, E. C. . . . p. 293

Ménaché, D. . . . p. 73

Merril, Carl R. . . . p. 137

Meyer, Barbara C. . . . p. 146, 165, 168, 170, 175

Meyer, Harry M., Jr. . . . p. 31, 123, 146, 155, 156, 159, 160, 168, 170, 172, 175, 177, Introduction

Milhorat, Thomas. . . . p. 121, 122

Miller, Carl E. . . . p. 26, 34, 266, 300, 309

Miller, J. Richard. . . . p. 83

Mitchell, Freddie. . . . p. 214

Morris, J. Anthony . . . p. 48, 50, 245, 250, 251, 299

Moy, Richard A. . . . p. 286

Mudd, S. Harvey. . . . p. 161, 164

Murray, Roderick. . . . p. 179, Introduction

Mushinski, J. F. . . . p. 75

Mustafa, Anne J. . . . p. 70, 71

Myers, Murrel L. . . . p. 200, 201

Nelson, John M. . . . p. 52, 56, Introduction

Neufeld, Elizabeth F. . . . p. 161, 164

Newman, J. T. . . . p. 52, 53, 55, 58

Nieman, Wendell. . . . p. 251

Norman, Phillip. . . . p. 13, 289

Norris, George F. . . . p. 200, 201

O'Brien, Thomas C. . . . p. 179, Introduction

O'Malley, Joseph P. . . . p. 299, 303

O'Neill, Hugh J. . . . p. 272

Oppenheim, Joost J. . . p. 46, 47, 195

Orr, Henry C. . . . p. 180, 214, 231, 233, 235, 237, 241, Introduction

Ozer, Harvey. . . . p. 117, 118, 119

Palmer, Amos E. . . . p. 52, 55, 113, 125, 204, 211, Introduction

Parkman, Paul. . . . p. 123, 146, 155, 156, 171, Introduction

Paul, Elizabeth B. . . . p. 67, 68

Pedreira, Frank. . . . p. 123, 125, 137, 200, 201, 209, 211, 283

Peters, Elizabeth. . . . p. 117, 118, 119

Petricciani, John. . . . p. 113, 117, 118, 130, 133, 135, 287, 288, Introduction

Peyton, M. P. . . . p. 73

Pisano, John J. . . . p. 70, 73

Pittman, Margaret. . . . p. 7, 18, 22, 26, 30, 31, 32, 36, 300, Introduction

Potter, Michael. . . . p. 74, 75

Prescott, Benjamin. . . . p. 293

Probst, Peter G. . . . p. 235

Purcell, Robert H. . . . p. 158

Rabson, Alan. . . . p. 117, 118

Reich, A. K. . . . p. 313, Introduction

Richardson, S. H. . . . p. 26, 27

Riggs, Donald B. . . . p. 48

Roberts, C. O. . . . p. 29

Robinson, Clarence A. . . . p. 83

Roelands, Joan. . . . p.67

Rogentine, Nicolas. . . . p. 157, 158

Rogers, Joseph L. . . . p. 231, 235

Rogers, N. . . . p. 198

Rosenblum, Edith N. . . . p. 157, 158, 169

Root, Richard K. . . . p. 9, 10

Rosen, Gerald. . . . p. 137

Rowan, Dighton. . . . p. 259

Ruddle, Frank. . . . p. 165, 166, 167, 278

Russell, Elmer C. . . . p. 99

Rusten, George. . . . p. 115

Sack, R. B. . . . p. 29

Sant-Agnese, Paul A. di. . . . p. 161

Saglam, Mehmet. . . . p. 251

Schlesinger, Edward R. . . . p. 268

Schlossman, S. F. . . . p. 239

Schofield, Frank. . . . p. 18

Schumacher, Heinz P. . . . p. 179, 197, Introduction

Seegmiller, J. E. . . . p. 164

Seligmann, Edward B., Jr. . . . p. 22, 39, 42, 43, 44, 83, 86, 87, 89, 91, 93, 97, 99, 101, 103, 105, 108, 123, 205, 207, 233, 285, 298, 299, 300, 311, Introduction

Shaffer, Margaret. . . . p. 305

Shaw, Colonel Eugene D. . . . p. 165

Sheagren, John N. . . . p. 42, 43

Shelokov, Alexis. . . . p. 125, 179, 193, 201, 202, 203, 204, 207, 211, 216, 217, 218, Introduction

Shulman, N. Raphael. . . . p. 59, 165

Simsarian, James P. . . . p. 146, 156, 175, Introduction

Singer, Stanley H. . . . p. 49, 113, 120, 122, 128, 129, 264, 268, 280, 282, 305, 306, Introduction

Sloan, Howard R. . . . p. 161, 164

Smith, Kendall O. . . . p. 52, 58, 59, 112, 118, 119, 123, 165, 297, 300, Introduction

Synder, Roger. . . . P. 197

Sorg, T. J. . . . p. 201

Spector, Sheldon L. . . . p. 207, 216, 297

Stang, Peter C. . . . p. 67, 68

Stanley, Walter. . . . p. 121, 122

Steenberg, R. W. . . . p. 29

Steinberg, D. . . . p. 164

Stevan, Lee J. . . . p. 87, 89, 105

Stevens, David P. . . . p. 130, 132, 159, 165

Stewart, G. L. . . . p. 177

Stokes, J. Jr. . . . p. 149

Swart, Barbara. . . . p. 307, 308

Szwed, Clarence F. . . . p. 67, 259

Takemoto, K. K. . . . p. 117, 118, 119

Tauraso, Nicola M. . . . p. 123, 125,179, 180, 191, 200, 201, 202, 204, 205, 207, 209, 211, 212, 213, 214, 216, 217, 219, 231, 241, 283, 300, 311, Introduction

Taylor, Mervin. . . . p. 70, 71

Thiel, John F. . . . p. 52, 53, 55, 58, 59

Thor, Daniel E. . . . p. 159, 179, 239, 241, 243, Introduction

Trousdale, Melvin D. . . . p. 52, 53, 59

Turner, Howard. . . . p. 268

Uhlendorf, B. William. . . . p. 161, 164

Valerio, David. . . . p. 283

Vasington, Paul. . . . p. 288

Verna, John E. . . . p. 48, 270, 290

Wagner, John C. . . . p. 311, Introduction

Walsh, John. . . . p. 157, 158

Weibel, R. E. . . . p. 149

Weiss, H. J. . . . p. 151

Weiss, Emilio. . . . p. 229

Wera, Thomas J. . . . p. 42, 44, Introduction

West, Joseph W. . . . p. 26, 83

Williams, Donna. . . . p. 67, 68

Wolff, Sheldon M. . . . p. 9, 10, 159, 243

Woodard, G. . . . p. 277

Wong, Vernon. . . . p. 30

Yamashiroya, Herbert M. . . . p. 48, 275

Young, Bobby G. . . . p. 270, 275, 277, 278, 307, 308

Young, Andrew M. . . . p. 67, 70, 71

Ziegler, John L. . . . p. 158

Ziring, P. R. . . . p. 151

INDEX TO PUBLICATIONS
Division of Biologics Standards
July 1, 1968 through June 30, 1969

Page

Abels, B. R., Gajdusek, D. C., Gibbs, C. J., Jr., Albrecht, P., & Rogers, N.
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Page

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Am. J. Epidem. (In press) 250

Brown, P., Gajdusek, D. C., & Morris, J. A.
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Science. (In press) 250

Carbone, P. P., Berard, C. W., Bennett, J. M., Ziegler, J. L., Cohen, M. H., & Gerber, P.
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Arch. Int. Med. (In press) 158

Carpenter, C. C. J., Sack, R. B., Feeley, J. C., & Steenberg, R. W.
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Page

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Page

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(In press) 59

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J. Clin. Invest. (In press) 164

Hirschman, R. J., Shulman, N. R., Barker, L. F., & Smith, K. O.
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JAMA (In press) 59

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Page

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J. Pediatrics 73:653-675, Nov., 1968 170

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Mental Retard. (In press) 155

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Page

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and complement fixing antigens.
Amer. J. Trop. Med. & Hyg. (In Press) 201

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Kirschstein, R. L. 125
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Proc. Soc. Exp. Biol. Med. 129:472-477, 1968 211

Pisano, J. J., Finlayson, J. S., & Peyton, M. P.
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Rogentine, N. G., & Gerber, P.
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Transplantation (In press) 158

Page

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Enhanced virus yields and decreased interferon production in mycoplasma infected hamster cells. 120, 129&
Proc. Soc. Exp. Biol. Med. (In Press) 306

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Enhanced vesicular stomatitis virus yields from mycoplasma infected hamster cells. 120, 129&
Nature (In press) 306

Singer, S. H., Kirschstein, R. L., & Baron, S. 119,
Viral inhibition of adenovirus 5 treated hamster cells. 129&
Proc. Soc. Exp. Biol. Med. 130:376-381, 1969 306

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Biochem. Biophys. Res. Comm. (In press) 164

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Proc. Soc. Exp. Biol. & Med. (In press) 59

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Avian Diseases (In press) 213

Page

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Appl. Microbiol. (In press) 207

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